Dissecting the Heterogeneity of Triple-Negative Breast Cancer

Otto Metzger-Filho, Andrew Tutt, Evandro de Azambuja, Kamal S. Saini, Giuseppe Viale, Sherene Loi, Ian Bradbury, Judith M. Bliss, Hatem A. Azim Jr, Paul Ellis, Angelo Di Leo, José Baselga, Christos Sotiriou, and Martine Piccart-Gebhart

ABSTRACT

Triple-negative breast cancer (TNBC) accounts for 15% to 20% of breast cancers. It is a heterogeneous disease, not only on the molecular level, but also on the pathologic and clinical levels. TNBC is associated with a significantly higher probability of relapse and poorer overall survival in the first few years after diagnosis when compared with other breast cancer subtypes. This is observed despite its usual high sensitivity to chemotherapy. In the advanced setting, responses observed with chemotherapy lack durability. Early-stage clinical studies suggested impressive potential when a poly (ADP-ribose) polymerase (PARP) inhibitor is given for the treatment of advanced TNBC with BRCA gene dysfunction. The molecular complexity of TNBC has led to proposed subclassifications, which will be of great value for the development of targeted therapies. In this review, we discuss the biology of TNBC at the pathologic and the molecular levels. We also elaborate on the role of systemic therapies and the results of the first phase III clinical trial evaluating the addition of iniparib, a novel investigational anticancer agent that does not possess characteristics typical of the PARP inhibitor class, in combination with chemotherapy in advanced TNBC.

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INTRODUCTION

Triple-negative breast cancer (TNBC) is defined by the lack of estrogen receptors (ERs) and progesterone receptors (PRs) and by human epidermal growth factor receptor 2 (HER2) —negative status and accounts for 15% to 20% of newly diagnosed breast cancer (BC) cases.1 It has a distinct epidemiology, histologic features, and clinical behavior. Defining TNBC through the absence of predictive biologic markers is suboptimal and could explain its increasingly recognized heterogeneity. Subclassifications of TNBC based on the presence of biomarkers, gene signatures, and BRCA dysfunction have been proposed, as detailed in Figure 1.

This article provides an overview of relevant clinical and translational research findings in the field of TNBC, aiming to translate relevant findings to clinical practice. Therapeutic developments and the utility of known cytotoxics, such as platinum, are discussed in the context of TNBC heterogeneity. Specific focus is put on a new class of targeted drugs with the ability to modulate the DNA damage repair machinery, namely the poly (ADP-ribose) polymerase (PARP) inhibitors. Iniparib, a novel investigational anticancer agent that does not possess characteristics typical of PARP inhibitors and for which investigations into its real mechanism of action are still ongoing, is also discussed.

References for this review were identified by conducting searches of Medline and selecting references from relevant articles using the terms “basal” or “triple negative” and “breast neoplasm” without restrictions for date. Only articles in English were used. Proceedings from conferences of the American Society of Clinical Oncology and San Antonio Breast Cancer Symposium were also manually searched for relevant abstracts.

Epidemiology and clinical characteristics

TNBC is associated with African American ethnicity,1,5 younger age,1,6,7 advanced stage at diagnosis, and poorer outcome when compared with other BC subtypes.8,11 Different population-based studies have demonstrated a higher prevalence of TNBC among women of African American or black ethnicity.1,5 A clear correlation has been made between young age at diagnosis and TNBC. In a large population-based study involving 6,370 patients, women with TNBC were significantly more likely to be under the age of 40 years.1 TNBC is known to have an early peak of recurrence between the first...
BC (84% first site of disease recurrence when compared with other types of patients, a greater proportion of TNBCs had visceral metastasis as attribution of recurrence. In a retrospective analysis of 1,608 patients with hormone receptor–positive BC (highly proliferative over the first 5 years, but with longer follow-up, the subset of patients with hormone receptor–positive BC (highly proliferative subset) had the same incidence of visceral metastasis as TNBC.

In clinical practice, patients are selected for treatment based on clinical stage, tumor histology, and biomarkers with the ability to predict response to treatment. HER2 receptor assessment follows a clinical stage, tumor histology, and biomarkers with the ability to predict response to treatment. HER2 receptor assessment follows a

HOW SHOULD TNBC BE DEFINED IN CLINICAL PRACTICE?

In clinical practice, patients are selected for treatment based on clinical stage, tumor histology, and biomarkers with the ability to predict response to treatment. HER2 receptor assessment follows a standardized definition according to guidelines, but hormone receptor assessment varies across different countries, and different immunohistochemistry (IHC) cutoffs are used to define positivity. The American Society of Clinical Oncology/College of American Pathologists guidelines for IHC testing for ERs and PRs recommend that ER and PR assays be considered positive if there is at least 1% of positive tumor cells in the sample. The adoption of a standardized definition for hormone receptor positivity worldwide would enable better definition of patients with TNBC and thus improve the quality of research conducted on this patient subset.

In this respect, the need for an accurate and reproducible assessment of triple-negative status by pathologists cannot be overemphasized. Every effort must be made to improve the accuracy and reproducibility of the assays by fostering compliance with the existing recommendations and guidelines. This will optimize the preanalytic and analytic phases of the testing procedures and the interpretation and scoring of the test results.

Although the definition of TNBC depends strongly on pathology, the term basal-like (B-L) BC is derived from gene expression studies. In their seminal article, Perou et al described distinct BC molecular subtypes, with gene expression patterns resembling luminal epithelial cells (luminal), basal and/or myoepithelial cells (B-L), and a subtype showing amplification of high expression of the Erb-B2 (HER2) gene. Further studies using independent data sets have shown similar clusters, with prognostic associations.

The complexity and costs of gene expression profiling limit its use in clinical practice. Different research groups have proposed IHC-based surrogates to diagnose the genomically defined B-L subtype. Basal cytokeratins (CKs; CK5/6 and/or CK17) correctly identified B-L BCs defined by gene expression profiling in early studies. The IHC variables most commonly used in IHC-based surrogates to identify the B-L subtype in subsequent studies were the triple negativity definition (ER negative, PR negative, and HER2 negative), basal CKs (ie, CK5/6, CK14, and CK17), epidermal growth factor receptor (EGFR), and C-kit (CD117). Nielsen et al evaluated 21 breast tumors defined as B-L by gene expression profiling and demonstrated CK5/6, EGFR, and C-kit expression in 62%, 57%, and 29%, respectively. Rakha et al subsequently proposed a similar IHC surrogate characterized as ER, PR, and HER2 negative and CK5/6, CK14, and CK17 positive, or EGFR positive. The presence of such a B-L surrogate within a group of TNBCs was associated with shorter survival when compared with the remaining TNBCs.

The heterogeneity in the staining patterns of CKs and the absence of defined cutoffs are factors limiting the use of such IHC-based surrogates. At present, there is no standardization for a panel of IHC markers to identify B-L cancers, limiting their applicability in clinical practice. The lack of a consensus definition for stratifying TNBCs into subtypes attests to the molecular complexity of basal tumors, underscoring the need for comprehensive translational research efforts in this field.

At the morphology level, TNBC and B-L tumors share similar characteristics. Larger tumor size, higher grade, presence of geographic necrosis, pushing borders of invasion, and stromal lymphocytic infiltrate are characteristics commonly reported across different series. The majority of TNBCs are invasive ductal carcinoma, but less common histologic subtypes (ie, medullary, metastatic, and adenoid cystic) share the TNBC phenotypic characteristics. However, caution should be used when stratifying risk among patients with TNBC with special histologic subtypes because tumor types such as classically defined medullary carcinoma and adenoid cystic carcinoma have an inherently favorable prognosis despite being classified as TNBCs.

An important phenotypic overlap is present between BRCA1-associated tumors and TNBC/B-L cancers. Initial attempts to classify BRCA1 mutation carriers at a genomic level classified all 18 BRCA1-mutant tumors as basal. IHC-based studies also classify 80% to 90% of BRCA1-associated tumors as TNBC and/or B-L. Subsequent studies have demonstrated the importance of BRCA1 dysfunction in this group of tumors, as further detailed later. In contrast to BRCA1, no association with TNBC is present for BRCA2 carriers.
Advances in the field of gene expression profiling have resulted in the development of different signatures aiming to provide better prognostic and predictive tools than classical clinicopathologic parameters. The first generation of signatures, including the 21-recurrence score,46 gene-70,40 genomic grade index,23 and others,47 was found to be useful for determining the risk of relapse in the ER-positive subgroup, yet was much less informative for basal and HER2-positive subtypes, which were assigned to the high-risk category in almost all cases.48

A recent effort to identify biologically distinct TNBC subgroups using the transcriptome data set from 21 independent BC studies identified different clusters defined by mesenchymal features, immune system–related genes, DNA damage response genes, and activated androgen receptor signaling.49 Interestingly, previous research groups have demonstrated the importance of individual components of these TNBC clusters.48,50-60

The first cluster, the claudin-low BC subtype, is characterized by mesenchymal features, low expression of cell-cell junction proteins (ie, claudin, E-cadherin), and intense immune infiltrate.50 Clinically the majority of claudin-low tumors are TNBCs. From a biologic perspective, the claudin-low subtype represents the most primitive tumors, on a scale of epithelial cell differentiation.51,52

With respect to the second cluster, different research groups have demonstrated that genes involved in the immune system can provide prognostic information about TNBC and ER-negative BC. A pooled analysis of microarray studies with more than 2,000 patients with BC observed that high expression of an immune response gene module was significantly associated with better outcome among patients with TNBC.48 Similar findings were observed among patients with ER-negative BC.54,55 An immune response seven-gene module54 and a 14-gene signature55 linked to immune/inflammatory chemokine regulation were capable of identifying a subgroup of patients with ER-negative BC with reduced risk of distant relapse. In addition, tumor lymphocyte infiltration was associated with better prognosis among patients with TNBC.61 Finally, immune-related metagenes have also shown ability to predict response to therapy; in the Trial of Principle (TOP) study where patients with ER-negative BC were treated with single-agent epirubicin, an immune response gene module was directly correlated with pathologic complete response (pCR).59

With regard to the third TNBC cluster, a gene signature based on DNA repair genes identified patients with TNBC responding to a neoadjuvant anthracycline, which could arguably be attractive for further validation in the context of prediction of response to DNA repair targeting agents.53 DNA damage response genes have direct implications for drug development as detailed later.

The final TNBC cluster identified highlights the importance of androgen signaling. Specifically, in silico experiments have demonstrated that a subset of TNBC has gene expression that closely matches that of ER-positive tumors, and this subset was found to have androgen receptor expression.60

As the heterogeneity of TNBC is better defined, potential therapeutic targets are likely to emerge. A better understanding of the immune system is likely to foster new therapies designed to modulate immune response. For the time being, studies in TNBC are focused on evaluating the role of novel cytotoxics or available cytotoxics in combination with known target agents, as detailed in the next section.

The lack of identified molecular targets in the majority of TNBCs implies that chemotherapy remains the treatment of choice for patients with TNBC. Neoadjuvant studies have shown that TNBC is highly chemotherapy sensitive.5,62-64 A retrospective analysis demonstrated twice the pCR rate in TNBC versus non-TNBC (22% vs 11%, respectively; odds ratio, 1.53; 95% CI, 1.03 to 2.26; \( P = .034 \)). Despite the high chemotherapy sensitivity, treatment of TNBC remains challenging, and on recurrence, patients with TNBC have worse survival outcomes than patients with hormone receptor–positive BC subtypes.65,66

In the adjuvant setting, no clear distinction can be made regarding the benefit of particular regimens according to BC subtypes. A decline in the use of anthracyclines for women with BC has been observed in the United States in the recent past.67 Nevertheless, anthracyclines remain an important class of drugs for treating TNBC. Retrospective exploratory analyses evaluating anthracycline benefit in patients with TNBC should be carefully evaluated. In the MA.5 phase III clinical trial, which compared cyclophosphamide, methotrexate, and fluorouracil (CMF) with cyclophosphamide, epirubicin, and fluorouracil (CEF), a superiority of CMF over CEF was demonstrated in a subset of 35 patients with B-L BC (IHC definition)68 and, subsequently, in a subset of 94 B-L tumors (reverse transcriptase polymerase chain reaction definition).69 However, the lack of a statistically significant interaction between treatment and B-L BC subtype and the small number of patients limit definitive conclusions.68,69 In a combined analysis of five adjuvant trials comparing anthracycline-containing regimens to CMF, anthracycline-containing regimens seemed to be more active than CMF in the TNBC subgroup.70 Moreover, when epirubicin was added to CMF versus CMF alone, the results of a randomized phase III study demonstrated superior 5-year disease-free survival (85% vs 59%, respectively; \( P = .002 \)) and 5-year overall survival (OS; 91% vs 73%, respectively; \( P = .002 \)) in patients with TNBC.71

With regard to the use of taxanes in the adjuvant setting, a meta-analysis has shown that the addition of a taxane to an anthracycline-based regimen improves disease-free survival and OS independently of ER expression.72 Hence, an anthracycline/taxane-based regimen currently seems to be the most suitable option for TNBC outside of the context of a clinical trial.

Ongoing adjuvant clinical trials aiming to improve the outcomes of patients with early-stage TNBC are listed in Table 1.73-77 The epothilone B analog ixabepilone, which has been shown to have activity in highly pretreated advanced TNBC, is being compared with classical taxanes.78 Exploratory analysis in a subset of 202 patients with TNBC enrolled onto the FinXX study showed improved efficacy when capecitabine was added to an anthracycline/taxane-based regimen.79 Capecitabine is also being evaluated as a maintenance therapy after standard adjuvant therapy in two phase III studies.74,75 However, the addition of capecitabine to a chemotherapy backbone without a comparator limits the evaluation of a specific interaction between capecitabine and TNBC.

In advanced TNBC, responses to chemotherapy lack durability. In a retrospective series of 3,726 patients with 14.8 years of median follow-up, the median survival of patients with metastatic TNBC was only 6 months.65 Bevacizumab, an anti–vascular endothelial growth
factor monoclonal antibody, was evaluated for the treatment of advanced BC across five phase III studies.\textsuperscript{86-88} The group analysis of data from the three first-line bevacizumab studies demonstrated progression-free survival (PFS) benefit in the subset of TNBC (PFS 4.7 v 10.2 months; hazard ratio [HR], 0.45; 95% CI, 0.33 to 0.61), but no OS gain.\textsuperscript{85} Bevacizumab is under evaluation in a large adjuvant phase III study, as detailed in Table 1.\textsuperscript{73} Another therapeutic option explored is the EGFR monoclonal antibody cetuximab, given the relatively high expression of EGFR in TNBC.\textsuperscript{86} In a phase II study, cetuximab in combination with carboplatin was associated with 18% overall response rate (ORR)\textsuperscript{87}. A parallel randomized phase II study compared cisplatin with cisplatin plus cetuximab.\textsuperscript{88} The combination arm was associated with increased ORR compared with cisplatin alone (20% v 10%, respectively; odds ratio, 2.13; 95% CI, 0.81 to 5.59; \textit{P} = .11).\textsuperscript{88} However, the evidence available about the role of EGFR as a driver of BC oncogenesis has not been convincing thus far. It is likely that multilevel downstream activation of EGFR and parallel signaling pathways may have reduced the efficacy of a single-target therapeutic approach.\textsuperscript{89,90}

Scientific evidence linking defective DNA repair machinery and sensitivity to DNA-damaging agents in TNBC has been considered as a potentially important factor that might influence therapeutic development. A number of clinical studies have evaluated the role of platinum salts in this population, as detailed in Table 2.\textsuperscript{91-97} In the subset of patients with \textit{BRCA} mutations, striking pCR rates have been demonstrated with single-agent cisplatin.\textsuperscript{91-95} However, the role of platinums in non–\textit{BRCA}-mutant advanced TNBC requires further validation. The Triple-Negative Breast Cancer Trial (TNT) is an ongoing randomized phase III study comparing carboplatin with docetaxel for the treatment of advanced TNBC.\textsuperscript{96} In addition, the Cancer and Leukemia Group B 40603 neoadjuvant study is evaluating weekly paclitaxel followed by dose-dense anthracycline-cyclophosphamide with or without the addition of carboplatin and/or bevacizumab in early TNBC.\textsuperscript{99} Results from both studies are awaited.

## DNA Damage Repair Modulation: PARP Inhibitors and Inparib

A range of DNA repair pathways are organized to maintain stability and integrity of the genome.\textsuperscript{100,101} Targeting mechanisms of DNA damage repair (DDR) is an innovative approach being developed for TNBC. Cancer cells are known to acquire DNA mutations over time, and failures in the mechanisms of DDR favor genetic instability and tumorigenesis.\textsuperscript{102} The remaining DNA repair mechanisms (those that were not lost during tumor progression) are upregulated and may be involved in resistance to DNA-damaging agents.

DNA repair mechanisms can be classified into categories repairing either single- or double-stranded damage. When one DNA strand is affected and the complementary strand is intact, direct

### Table 1. Ongoing Adjuvant Phase III Clinical Trials for the Treatment of Triple-Negative Breast Cancer

<table>
<thead>
<tr>
<th>Study Design</th>
<th>ClinicalTrials.gov Identifier</th>
<th>Estimated Enrollment (No. of Patients)</th>
<th>Study Design</th>
<th>Treatment</th>
<th>Study Design</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEATRICE NCT00529687\textsuperscript{73}</td>
<td>4,430</td>
<td>adj ct + bev (\rightarrow) bev up to 1 year v adj ct</td>
<td>BEATRICE NCT00529687\textsuperscript{73}</td>
<td>adj ct + bev (\rightarrow) bev up to 1 year v adj ct</td>
<td>BEATRICE NCT00529687\textsuperscript{73}</td>
<td>adj ct + bev (\rightarrow) bev up to 1 year v adj ct</td>
</tr>
<tr>
<td>CIBOMA NCT00130533\textsuperscript{74}</td>
<td>876</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
<td>CIBOMA NCT00130533\textsuperscript{74}</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
<td>CIBOMA NCT00130533\textsuperscript{74}</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
</tr>
<tr>
<td>SYSCBS-001 NCT01112826\textsuperscript{75}</td>
<td>684</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
<td>SYSCBS-001 NCT01112826\textsuperscript{75}</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
<td>SYSCBS-001 NCT01112826\textsuperscript{75}</td>
<td>adj ct (\rightarrow) capcetabine 1 year v adj ct</td>
</tr>
<tr>
<td>TITAN NCT00789581\textsuperscript{76}</td>
<td>1,800</td>
<td>AC (\times) 4 (\rightarrow) ixabepilone (\times) 4 v AC (\times) 4 (\rightarrow) paclitaxel every week (\times) 12</td>
<td>TITAN NCT00789581\textsuperscript{76}</td>
<td>AC (\times) 4 (\rightarrow) ixabepilone (\times) 4 v AC (\times) 4 (\rightarrow) paclitaxel every week (\times) 12</td>
<td>TITAN NCT00789581\textsuperscript{76}</td>
<td>AC (\times) 4 (\rightarrow) ixabepilone (\times) 4 v AC (\times) 4 (\rightarrow) paclitaxel every week (\times) 12</td>
</tr>
<tr>
<td>PACS08 NCT00630032\textsuperscript{77}</td>
<td>2,500</td>
<td>FEC (\times) 3 (\rightarrow) ixabepilone v FEC (\times) 3 (\rightarrow) docetaxel</td>
<td>PACS08 NCT00630032\textsuperscript{77}</td>
<td>FEC (\times) 3 (\rightarrow) ixabepilone v FEC (\times) 3 (\rightarrow) docetaxel</td>
<td>PACS08 NCT00630032\textsuperscript{77}</td>
<td>FEC (\times) 3 (\rightarrow) ixabepilone v FEC (\times) 3 (\rightarrow) docetaxel</td>
</tr>
</tbody>
</table>

**Abbreviations:** AC, anthracycline cyclophosphamide; adj ct, adjuvant chemotherapy; BEATRICE, A Study of Avastin (Bevacizumab) Adjuvant Therapy in Triple-Negative Breast Cancer; bev, bevacizumab; CIBOMA, Iberoamerican Coalition for Breast Oncology Research; FEC, fluorouracil, epirubicin, and cyclophosphamide; PACS08, Combination Chemotherapy Followed by Docetaxel or Ixabepilone in Treating Patients Who Have Undergone Surgery for Nonmetastatic Breast Cancer; SYSCBS-001, Efficacy of Capecitabine Metronomic Chemotherapy to Triple-Negative Breast Cancer; TITAN, Randomized Trial of Ixabepilone Versus Taxol in Adjuvant Therapy of Triple-Negative Breast Cancer.

### Table 2. Reported Studies Evaluating Cisplatin or Carboplatin for the Treatment of Patients With \textit{BRCA}-Mutant Breast Cancer and/or TNBC

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Population</th>
<th>No. of Patients</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhattacharya et al\textsuperscript{96}</td>
<td>Randomized phase II</td>
<td>TNBC</td>
<td>126</td>
<td>Metronomic CM (n = 66) v metronomic CM + cisplatin (n = 60)</td>
<td>ORR: 62% (95% CI, 47.5% to 77%); ORR: metronomic CM + cisplatin, 62%</td>
</tr>
<tr>
<td>Wang et al\textsuperscript{97}</td>
<td>First-line advanced TNBC</td>
<td>45</td>
<td>Cisplatin + gem</td>
<td>ORR: 62% (95% CI, 47.5% to 77%); ORR: metronomic CM + cisplatin, 62%</td>
<td></td>
</tr>
<tr>
<td>Silver et al\textsuperscript{94}</td>
<td>Neoadjuvant phase II</td>
<td>TNBC, n = 2 \textit{BRCA1} mutant</td>
<td>28</td>
<td>Cisplatin</td>
<td>pCR: 22% (95% CI, 9% to 43%)</td>
</tr>
<tr>
<td>Gronwald et al\textsuperscript{91}</td>
<td>Neoadjuvant phase II</td>
<td>\textit{BRCA1} mutant</td>
<td>25</td>
<td>Cisplatin</td>
<td>pCR: 72%</td>
</tr>
<tr>
<td>Alba et al\textsuperscript{92}</td>
<td>Neoadjuvant randomized phase II</td>
<td>TNBC</td>
<td>94</td>
<td>EC (\times) 4 (\rightarrow) T v EC (\times) 4 (\rightarrow) T + carbo</td>
<td>pCR: EC (\times) 4 (\rightarrow) T, 30%; EC (\times) 4 (\rightarrow) T + carbo, 30%</td>
</tr>
<tr>
<td>Byrski et al\textsuperscript{93}</td>
<td>Retrospective-neoadjuvant</td>
<td>\textit{BRCA1} mutant</td>
<td>102</td>
<td>CMF, n = 14; AC, n = 23; FAC, n = 28; pCR: CMF, 7%; AC, 22%; FAC, 21%; AT, 8%; cisplatin, 83%</td>
<td></td>
</tr>
<tr>
<td>Byrski et al\textsuperscript{93}</td>
<td>Retrospective-neoadjuvant</td>
<td>\textit{BRCA1} mutant</td>
<td>10</td>
<td>Cisplatin</td>
<td>pCR: 90%</td>
</tr>
<tr>
<td>Gronwald et al\textsuperscript{91}</td>
<td>Neoadjuvant phase II</td>
<td>\textit{BRCA1} mutant</td>
<td>23; FAC, n = 28; cisplatin, n = 12</td>
<td>pCR: CMF, 7%; AC, 22%; FAC, 21%; AT, 8%; cisplatin, 83%</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** A, doxorubicin; C, cyclophosphamide; carbo, carboplatin; E, epirubicin; F, fluorouracil; gem, gemcitabine; M, methotrexate; ORR, overall response rate; pCR, pathologic complete response; T, docetaxel; TNBC, triple-negative breast cancer.
repair, base excision repair, nucleotide excision repair, and mismatch repair are activated to correct it. For damage leading to breaks in both DNA strands (double-stranded breaks [DSBs]), the following two main repair pathways are available: nonhomologous end joining, which can induce mutagenic deletion or inappropriate rejoining between DSBs, and the potentially more accurate homologous recombination repair.

Deficiencies in the BRCA1 gene pathway are important for understanding the sensitivity of drugs targeting DDR in TNBC. The BRCA1 gene is essential for maintaining genomic stability by promoting repair of DSBs, particularly where these arise at arrested DNA replication forks.41 The majority of BCs arising in the most abundant.114 PARP1 and PARP2 are involved in the mechanism of DNA damage (and potentiates cell cycle effects of chemotherapy in tumor cell lines.126 However, the molecular mechanism accounting for the observed cellular effects has yet to be elucidated, and the relevance of these cellular effects for clinical anticancer activity is not known. In this regard, O’Shaughnessy et al121 have conducted a randomized phase II trial in which a total of 123 patients with locally defined TNBC (<10% ER/PR immunoreactive cells and HER2 negative) were randomly assigned to receive the combination of gemcitabine and carboplatin (GC) or GC plus iniparib (GCI) but were allowed to cross over on centrally confirmed progression of disease. GCI, compared with GC, resulted in an increased clinical benefit rate (56% v 34%, respectively; P = .01), ORR (52% v 32%, respectively; P = .02), PFS (5.9 v 3.6 months, respectively; HR, 0.59; P = .01), and OS (12.3 v 7.7 months, respectively; HR, 0.57; P = .01). This was achieved with no significant increase in the rate of adverse events.

The same group of researchers then conducted a phase III study with PFS and OS as coprimary end points and randomly assigned 519 patients with TNBC to GCI or GC. The coprimary end points of PFS (HR, 0.79; 95% CI, 0.65 to 0.98; P = .027 [prespecified P for significance = .01]) and OS (HR, 0.88; 95% CI, 0.69 to 1.21; P = .28 [prespecified P for significance = .04]) suggested modest effects for GCI but did not reach the level of statistical significance prespecified in the trial’s analysis plan.122 The fact that the PFS and OS benefit for GCI was restricted to patients treated in second and third line is likely to be misleading because of the significant imbalance in various baseline characteristics; moreover, the differences in estimated treatment effect size between patients with first-line and second/third-line treatment seem less extreme once appropriate adjustment has been made for these factors in the multivariate analysis. For example, the disease-free interval in the first-line treatment strata has a shorter time from diagnosis to metastasis in the GCI arm versus GC arm.

### Table 3. PARP Inhibitors and Phase of Development

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Phase of Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iniparib (BSI-201)</td>
<td>BiPart/Novartis</td>
<td>Preclinical</td>
</tr>
<tr>
<td>BSI-401</td>
<td>BiPart/Novartis</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Olaparib (AZD2281)</td>
<td>KuDOS/AstraZeneca</td>
<td>III</td>
</tr>
<tr>
<td>Veliparib (IBT-888)</td>
<td>Abbot</td>
<td>II</td>
</tr>
<tr>
<td>CO-338</td>
<td>Clovis</td>
<td>II</td>
</tr>
<tr>
<td>INO-1001</td>
<td>Inotek</td>
<td>II</td>
</tr>
<tr>
<td>CEP-9722</td>
<td>Cephalon</td>
<td>I</td>
</tr>
<tr>
<td>MK-4827</td>
<td>Merck</td>
<td>II</td>
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<tr>
<td>E7016</td>
<td>Eisai</td>
<td>I</td>
</tr>
<tr>
<td>BMN-673</td>
<td>BioMarin</td>
<td>I</td>
</tr>
</tbody>
</table>

**NOTE.** Recent preclinical and clinical data indicate that iniparib does not possess characteristics typical of PARP inhibitor class.

Abbreviation: PARP, poly (ADP-ribose) polymerase.
treated with veliparib, olaparib, or iniparib, DSBs increased in a dose-

findings have shown that when a information about differences among PARP inhibitors and clarifica-

ever, there is still a critical need to identify patients without milestone in managing patients with

The results obtained with PARP inhibitors so far represent a real milestone in managing patients with BRCA-associated TNBC. However, there is still a critical need to identify patients without BRCA mutations likely to benefit from PARP inhibitors. In addition, more information about differences among PARP inhibitors and clarification of the exact mechanism of action of iniparib are needed. In vitro findings have shown that when a BRCA1/2-defective BC cell line was treated with veliparib, olaparib, or iniparib, DSBs increased in a dose-

and time-dependent fashion. However, only veliparib and olaparib were able to inhibit PARP1/2. In contrast, iniparib was able to suppress genes involved in telomere function, which the authors suggest may be a result of blockade of other PARP family members.

Table 4. Reported Results of Phase II and Phase III Studies With PARP Inhibitors in Breast Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Population</th>
<th>Treatment Regimens</th>
<th>Efficacy</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tutt et al</td>
<td>Phase II, two cohorts</td>
<td>54 patients with BC with ≥ 1 CT regimen; all with BRCA1/2 mutation</td>
<td>Olaparib 400 mg twice daily PO, every 28 days, n = 27; Olaparib 100 mg twice daily PO, every 28 days, n = 27</td>
<td>Olaparib 400 mg: RR, 41% (95% CI, 25% to 59%); CR, 4% (95% CI, 1% to 18%); PR, 37% (95% CI, 22% to 56%); SD, 44% (95% CI, 28% to 63%)</td>
<td>Grade 3 or 4: olaparib 400 mg: nausea, 15%; vomiting, 11%; fatigue, 15%; anemia, 11%; olaparib 100 mg: nausea, 0%; vomiting, 0%; fatigue, 4%; anemia, 7%</td>
</tr>
<tr>
<td>Isaioff et al</td>
<td>Phase II, single arm</td>
<td>41 patient with BC with ≥ 1 CT regimen; 8 patient with BRCA1/2 mutation (efficacy results)</td>
<td>TMZ 150 mg/m² PO on days 1-5; veliparib 40 mg twice daily PO on days 1-7, every 28 days (dose reduced to 30 mg twice daily)</td>
<td>ORR, 37%; CBR, 62%; PFS, 5.5 months</td>
<td>Grade 3: thrombocytopenia, 22%; neutropenia, 19%; hypophosphatemia, 2%</td>
</tr>
<tr>
<td>O'Shaughnessy et al</td>
<td>Phase II, randomized</td>
<td>123 patients with TNBC with ≥ 2 CT regimens, BRCA unknown</td>
<td>Carboplatin AUC 2 IV days 1 and 8; gemcitabine 1,000 mg/m² IV days 1 and 8, every 21 days, n = 62</td>
<td>Carboplatin/gemcitabine: ORR, 32%; CBR, 34%; PFS, 3.6 months (95% CI, 2.6 to 5.2 months); OS, 7.7 months (95% CI, 6.5 to 13.3 months)</td>
<td>Carboplatin/gemcitabine: grade 3: neutropenia, 36%; anemia, 15%; thrombocytopenia, 10%; fatigue, 17%</td>
</tr>
<tr>
<td>O'Shaughnessy et al</td>
<td>Phase III, randomized</td>
<td>519 patients with TNBC with ≥ 2 CT regimens; 57% first line; 43% second or third line; BRCA unknown</td>
<td>Carboplatin AUC 2 IV days 1 and 8; gemcitabine 1,000 mg/m² IV days 1 and 8, every 21 days, n = 258</td>
<td>Carboplatin/gemcitabine: ORR, 30% (95% CI, 25% to 35%); CBR, 30%; PFS, 4.1 months (95% CI, 3.1 to 4.6 months); OS, 11.1 months (95% CI, 9.2 to 12.1 months)</td>
<td>Carboplatin/gemcitabine: grade 3 or 4: neutropenia, 53%; anemia, 22%; thrombocytopenia, 24%; fatigue, 6%</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the concentration-time curve; BC, breast cancer; CBR, clinical benefit rate; CR, complete response; CT, chemotherapy; IV, intravenously; ORR, overall response rate; OS, overall survival; PARP, poly (ADP-ribose) polymerase; PFS, progression-free survival; PO, oral; PR, partial response; RR, objective response rate; SD, stable disease; TMZ, temozolomide; TNBC, triple-negative breast cancer.

TNBC is a challenging disease that has lacked a standardized treatment approach both in the early and advanced settings. Available evidence suggests that among patients with TNBC, prognosis seems to vary according to factors such as age and pathologic subtype. Several research groups have provided important insights into TNBC heterogeneity. Genes related to immune response have been shown to be of prognostic and predictive value, but validation is needed. PARP inhibitors have demonstrated impressive results in studies in the BRCA1/2 BC subpopulation, but the identification of nonmutant TNBC likely

CONCLUSION

(median, 9.5 v 15.9 months, respectively). In concordance with phase II data, no increase in grade 3 or 4 toxicities was observed.

Despite this trial’s failure to meet the prespecified statistical criteria for the use of coprimary end points, it did show a signal of efficacy for GCI within this heterogeneous group of patients. What is not clear is where the signal is coming from within the population and whether this relates to the degree of prior treatment or a biologic subgroup. The relatively limited understanding of the mechanism of action of iniparib currently compounds the challenges associated with resolving this issue. The results obtained with PARP inhibitors so far represent a real milestone in managing patients with BRCA-associated TNBC. However, there is still a critical need to identify patients without BRCA mutations likely to benefit from PARP inhibitors. In addition, more information about differences among PARP inhibitors and clarification of the exact mechanism of action of iniparib are needed. In vitro findings have shown that when a BRCA1/2-defective BC cell line was treated with veliparib, olaparib, or iniparib, DSBs increased in a dose-
to derive the same magnitude of benefit remains challenging. Prospective clinical trials coupled with integrated adequately powered translational research questions are likely to improve the outcome of patients with TNBC and should be our priority.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

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**REFERENCES**


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**Data analysis and interpretation:** Ian Bradbury, Judith M. Bliss, Otto Metzger-Filho

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Heterogeneity of Triple-Negative Breast Cancer


99. Clinical Trials.gov: Paclitaxel with or without carboplatin and/or bevacizumab followed by doxorubicin and cyclophosphamide in treating patients with breast cancer that can be removed by surgery. http://clinicaltrial.gov/ct2/show/NCT00861705?term=NCT00861705&rank=1


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