Targeting triple-negative breast cancer: optimising therapeutic outcomes

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Received 1 November 2011; revised 24 January 2012; accepted 8 February 2012

Background: Triple-negative breast cancer (TNBC) is a distinct subset of breast cancer (BC) defined by the lack of immunohistochemical expression of the estrogen and progesterone receptors and human epidermal growth factor receptor 2. It is highly heterogeneous and displays overlapping characteristics with both basal-like and BC susceptibility gene 1 and 2 mutant BCs. This review evaluates the activity of emerging targeted agents in TNBC.

Design: A systematic review of PubMed and conference databases was carried out to identify randomised clinical trials reporting outcomes in women with TNBC treated with targeted and platinum-based therapies.

Results and Discussion: Our review identified TNBC studies of agents with different mechanisms of action, including induction of synthetic lethality and inhibition of angiogenesis, growth, and survival pathways. Combining targeted agents with chemotherapy in TNBC produced only modest gains in progression-free survival, and had little impact on survival. Six TNBC subgroups have been identified and found to differentially respond to specific targeted agents. The use of biological preselection to guide therapy will improve therapeutic indices in target-bearing populations.

Conclusion: Ongoing clinical trials of targeted agents in unselected TNBC populations have yet to produce substantial improvements in outcomes, and advancements will depend on their development in target-selected populations.

Key words: basal like, breast cancer, cancer treatment, targeted therapy, triple negative, systematic review

Introduction

Prior to the advent of molecularly targeted therapies, unselected breast cancer (BC) populations were treated with chemotherapeutics acting indiscriminately on rapidly dividing cells. Targeted agents, including tamoxifen and trastuzumab (Herceptin®; Genentech), interfere with molecular pathways driving tumour growth and progression and were developed for use in subsets of patients expressing relevant biomarkers. Utilisation of targeted therapies in biologically preselected patient populations has significantly improved BC survival.

The term triple-negative breast cancer (TNBC) is used to describe tumours that are estrogen and progesterone receptor negative and human epidermal growth factor receptor 2 (HER2) normal, and accounts for ∼15%–20% of BC patients. This is confusing terminology, as TNBC reflects a heterogeneous population (Figure 1) [1, 2] with a more complex molecular transcriptome than is suggested by the triple-negative (TN) immunohistochemical (IHC) expression. The classification of breast tumours by gene expression signature revealed distinct subtypes with important implications for outcome [3, 4]. Of these subtypes, which include HER2-enriched, luminal A, luminal B, and basal-like breast cancer (BLBC; supplemental Table S1, available at Annals of Oncology online), BLBC has the poorest prognosis [5]. Gene expression analysis demonstrates that the molecular signature of TNBC generally overlaps with BLBC, with concordance rates of ∼70%–90% [6–8]. However, not all TNBC can be defined as BLBC [5, 9], as a small minority of BLBC patients do, in fact, have some estrogen receptor and HER2 expression.

TNBC is also associated with BRCA-related BC (supplemental Table S1, available at Annals of Oncology online), although the incidence of BRCA mutations in TNBC varies from 16% to 42% [10, 11]. Other mechanisms resulting in downregulation of BRCA1/2, including epigenetic alterations and overexpression of BRCA1 inhibitors [12–16], are also
Molecular heterogeneity of TNBC subtypes [1, 2]. These TNBC subgroups can be assigned to three intrinsic molecular subtypes: basal like (BL), dark shades of grey; BL1, BL2 and IM), mesenchymal like (light shades of grey: M and MSL) and luminal like (intermediate shade of grey: LAR). BL1, basal like 1; BL2, basal like 2; IM, immunomodulatory; LAR, luminal AR; M, mesenchymal; MSL, mesenchymal stem like.

associated with TNBC and likely contribute to the aneuploidy and genomic instability characteristic of this subgroup.

Chemotherapy remains the core treatment option for patients with TNBC. However, treatment with cytotoxic chemotherapy produces mixed results and has a variable impact on long-term prognosis [17–20]. In the neoadjuvant setting, TNBC exhibits a better response to chemotherapy compared with non-TNBC [21]. Paradoxically, those patients who do not respond well to preoperative chemotherapy have a high risk of relapse within the first 2 years and worse overall survival (OS) [17, 22–25] (3-year survival, 68% versus 94%, pathological complete response [pCR] non-responders versus responders) [21]. Metastatic TNBC is an aggressive disease that is associated with a high proliferation index [26], visceral and central nervous system metastases [21, 27, 28], and poor outcome despite treatment. Median survival of advanced TNBC is at best 12 months, much shorter than the duration of survival seen in other subtypes of advanced BC. Therefore, identification of specific targeted and more active therapies for TNBC patients remains an important clinical challenge.

With analysis of the complex gene expression pattern of TNBC, a multitude of molecular markers that may serve as viable targets for specific therapeutic intervention have been identified, including growth factor receptors, protein kinases, DNA repair enzymes, and tumour suppressor genes. This review identifies studies specifically testing targeted and platinum-based therapies for TNBC, and assesses the impact of emerging targeted therapies on treatment outcomes in TNBC.

**methods**

PubMed (through 3 June 2011), the American Society of Clinical Oncology (2010 and 2011), and San Antonio Breast Cancer Symposium (2009 and 2010) databases were searched at the title and abstract levels using the search terms ‘triple negative’ or ‘TNBC’ to identify studies investigating targeted therapy and/or platinum-based agents in TNBC. To ensure that TNBC sub-group analyses were identified, a bibliographic review of selected review articles (January 2010 to June 2011) was conducted.

A total of 1018 non-duplicate records were screened at the title and abstract levels to identify 43 reports of randomised trials in TNBC (supplemental Figure S1, available at *Annals of Oncology* online). Of these, 17 targeted trials and three platinum-based studies reported TNBC outcomes at the full text or presentation level. Ongoing randomised clinical trials of targeted therapy and platinum-based agents were identified by searching the ClinicalTrials.gov database on 12 July 2011 for ‘triple-negative’, ‘TNBC’, or specific agents (supplemental Figure S2, available at *Annals of Oncology* online).

**platinum-based agents and poly(ADP-ribose) polymerase1/2-inhibitors**

The frequency of *BRCA1/2* germline and somatic mutations in unselected TNBC populations is 20% [11], and germline frequency ranges from 11% to 39% in selected populations [5, 11, 29, 30]. As *BRCA1/2* are critical regulators of DNA repair and maintenance of genomic stability [31, 32], it was hypothesised that TNBC may be particularly sensitive to agents that cause DNA damage, including platinum-containing compounds that induce synthetic lethality in repair-defective cells via inhibition of poly(ADP-ribose) polymerase (PARP)1/2 pathways [33, 34]. Several clinical studies have investigated DNA-damaging agents, such as platinum-based regimens (Table 1 and supplemental Tables S2 and S3, available at *Annals of Oncology* online) and PARP inhibitors (Table 1), for the treatment of patients with TNBC.

**platinum salts**

Outcomes for platinum-containing agents administered as monotherapy for metastatic TNBC have been modest [41]. Platinum doublet or triplet therapy appears more active (supplemental Table S2, available at *Annals of Oncology* online) [38, 39, 42, 59], and three randomised trials have investigated the benefits of these combinations in TNBC (Table 1 and supplemental Table S3, available at *Annals of Oncology* online) [35, 36, 40]. In a randomised phase II trial (*N* = 126), the addition of cisplatin to metronomic m ethotrexate and cyclophosphamide resulted in an improvement in median time-to-progression (TTP) of 6 months (from 7 to 13 months) and OS of 4 months (from 12 to 16 months) in second-line metastatic TNBC patients [35]. The toxicity profile was manageable and, although promising, results were limited to a small single institutional setting.

In advanced TNBC, platinum have been combined with targeted agents such as bevacizumab (Avastin®; Roche-Genentech), cetuximab (Erbitux®; ImClone), erlotinib (Tarceva®; Roche-Genentech), and iniparib (sanoﬁ-aventis, supplemental Table S2, available at *Annals of Oncology* online) [31, 36, 43–47]. A randomised phase II trial demonstrated promising overall response rates (ORR) (17% versus 6%) when carboplatin was added to single-agent cetuximab in pretreated advanced TNBC patients [36], although progression-free survival (PFS) and OS outcomes are pending.

In the neoadjuvant setting, the addition of platinum agents to anthracycline and/or taxane regimens has shown promising outcomes, with pCRs ranging from 30% to 62% (supplemental Table S2, available at *Annals of Oncology* online) [40, 48–51] in phase II trials. The combination of weekly cisplatin added to weekly epirubicin and paclitaxel produced a pCR rate of 62% and 5-year disease-free survival (DFS) and OS values of 76% and 89%, respectively [48]. However, despite the promise of platinum combinations in TNBC, the addition of carboplatin...
Table 1 Randomized clinical trials evaluating platinum-derivatives or PARP inhibitors in advanced triple-negative breast cancer

<table>
<thead>
<tr>
<th>Trial</th>
<th>Line of treatment</th>
<th>Treatment algorithm</th>
<th>TNBC patients (N)</th>
<th>Median age, years (range)</th>
<th>ORR (%)</th>
<th>Median PFS (months); HR [95% CI]</th>
<th>Median OS (months); HR [95% CI]</th>
</tr>
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<tbody>
<tr>
<td><strong>Platinum derivatives</strong></td>
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<tr>
<td>Bhattacharyya [35],</td>
<td>Second-line</td>
<td>Cyclophosphamide 50 mg/d; methotrexate 2.5 mg BID, d1, 2, qw; cisplatin 20 mg/m² d1, qw</td>
<td>66</td>
<td>58 (38–72)</td>
<td>62</td>
<td>13 (TTP)</td>
<td>16</td>
</tr>
<tr>
<td>Rd phase II</td>
<td></td>
<td>Cyclophosphamide 50 mg/d; methotrexate 2.5 mg BID, d1, 2, qw</td>
<td>60</td>
<td>33</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Carey [36], TBCRC 001,</td>
<td>First-line+</td>
<td>Cetuximab 400 mg/m² initial dose; then 250 mg/m² qw; carboplatin AUC 2 qw (3 of 4 weeks)</td>
<td>71</td>
<td>51 (NR)</td>
<td>17</td>
<td>2 (entire cohort)</td>
<td>12 (entire cohort)</td>
</tr>
<tr>
<td>Rd phase II</td>
<td>(0–2 prior regimens)</td>
<td>Cetuximab 400 mg/m² initial dose; then 250 mg/m² qw</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>O'Shaughnessy [31],</td>
<td>First-line+</td>
<td>Gemcitabine 1000 mg/m² d1, 8, q3w; carboplatin AUC 2 d1, 8, q3w; iniparib 5.6 mg/kg d1, 8, 11, q3w</td>
<td>61</td>
<td>56 (34–76)</td>
<td>52 (P = 0.02)</td>
<td>5.9; 0.59 [0.39–0.90] (P = 0.01)</td>
<td>12.3; 0.57 [0.36–0.90] (P = 0.01)</td>
</tr>
<tr>
<td>Rd phase II</td>
<td>(0–3 prior regimens)</td>
<td>Gemcitabine 1000 mg/m² d1, 8, q3w; carboplatin AUC 2 d1, 8, q3w</td>
<td>62</td>
<td>53 (26–80)</td>
<td>32</td>
<td>3.6</td>
<td>7.7</td>
</tr>
<tr>
<td>O'Shaughnessy [37],</td>
<td>First-line+</td>
<td>Gemcitabine 1000 mg/m² d1, 8, q3w; carboplatin AUC 2 d1, 8, q3w; iniparib 5.6 mg/kg d1, 8, 11, q3w</td>
<td>261</td>
<td>53</td>
<td>34</td>
<td>5.1; 0.79 [0.65–0.98] (P = 0.027)</td>
<td>11.8; 0.88 [0.69–1.12] (P = 0.28)</td>
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<tr>
<td>Rd phase III</td>
<td>(0–2 prior regimens)</td>
<td>Gemcitabine 1000 mg/m² d1, 8, q3w; carboplatin AUC 2 d1, 8, q3w</td>
<td>258</td>
<td>54</td>
<td>30</td>
<td>4.1</td>
<td>11.1</td>
</tr>
</tbody>
</table>

* Crossover to cetuximab + carboplatin after progressive disease.
* Twenty-four of these patients crossed over to cetuximab + carboplatin.
* Approximated value reported.
* Crossover allowed upon progressive disease, 30 of 59 patients (51%) in the chemotherapy-alone group crossed over to receive iniparib in combination with gemcitabine and carboplatin.
* Crossover allowed upon progressive disease, 152 (96%) of progressing patients in the chemotherapy-alone group crossed over to receive iniparib in combination with gemcitabine and carboplatin at primary analysis.
* Prespecified alpha = 0.01.
* Prespecified alpha = 0.04.

AUC, area under the curve; BID, bi-daily; HR, hazard ratio; d, day; N, number of patients; NR, not reported; ORR, overall response rate; OS, overall survival; PARP, poly(ADP-ribose) polymerase; PFS, progression-free survival; PR, partial response; qXw, every X weeks; Rd, randomized; TNBC, triple-negative breast cancer; TTP, time-to-progression.
to anthracycline-taxane therapy did not improve pCR rates or rates of breast conserving surgery in BLBC patients enrolled in The Spanish Breast Cancer Research Group (GEICAM) 2006-03 phase II randomised trial (supplemental Table S3, available at Annals of Oncology online) [40]. Differential outcomes to platinum-based therapy may be explained by the potential inferiority of carboplatin relative to cisplatin, an issue apparent in other tumour types [52, 53], the heterogeneity of TNBC, or the lack of activity of platinum agents in TNBC. Platinum-derivative activity will be further addressed in three randomised trials, evaluating the addition of platinum derivatives to standard adjuvant therapy (NCT01150513, NCT01216111, NCR01378533; supplemental Figure S2, available at Annals of Oncology online).

**PARP inhibition**

Phase I and II studies of the PARP inhibitor olaparib (AZD2281; AstraZeneca) and veliparib (ABT-888; Abbott) have demonstrated promising activity in advanced BRCA mutation-associated BC of any subtype [54–56]. Olaparib activity (ORR of 54%) was seen in a small cohort (N = 13) of BRCA mutation-associated TNBC patients [55]; however, correlative research found no Response Evaluation Criteria in Solid Tumours confirmed activity (ORR; 0%, N = 15) in a small cohort of BRCA mutation-negative TNBC patients [57], and efforts to maintain the dose intensity of combinations of olaparib and paclitaxel in advanced TNBC have been frustrated by myelosuppression [58].

Iniparib has demonstrated activity in combination with chemotherapy (Table 1) [31]. Results of a randomised phase II trial combining iniparib with gemcitabine and carboplatin for the treatment of TNBC demonstrated a 41% reduction in the risk of progression (PFS; median, 3.6 versus 5.9 months; P = 0.01) and a 43% reduction in the risk of death (OS; median, 7.7 versus 12.3 months; P = 0.01; Table 1), with a minimal increase in toxicity [31]. Results of a multicenter, phase III trial assessing the same iniparib combination in advanced TNBC failed to meet the primary study end points [PFS, 4.1 versus 5.1 months; hazard ratio (HR) = 0.79 [95% confidence interval (CI) 0.65–0.98], P = 0.027; prespecified alpha = 0.01 and OS 11.1 versus 11.8 months; HR = 0.88 [95% CI 0.69–1.12], P = 0.28; prespecified alpha = 0.04; Table 1] [37]. The failure of the phase III iniparib trial to meet the prespecified criteria for significance may be due to a lack of power resulting from the selection of the co-primary end points of PFS and OS, as well as the questionable uniformity of the overall TNBC population and the BRCA subgroups. Whatever the reason, these findings have left clinicians confused regarding the benefits of iniparib [37].

Although PARP inhibitors have demonstrated activity in BRCA1/2 mutant-positive BC, they have not proven to be of significant benefit in unselected TNBC populations, as single agents [55] or in combination with standard chemotherapeutic agents [37, 58, 60–63], suggesting that the clinical utility of PARP inhibitors in unselected patients is uncertain. Ongoing clinical trials will further evaluate the benefits of weekly or biweekly iniparib in combination with gemcitabine and carboplatin in the advanced setting (NCT01045304), iniparib and PF-01367338 in the neoadjuvant and adjuvant settings (NCT01204125, NCT01074970), respectively (supplemental Figure S2, available at Annals of Oncology online), as well as other promising PARP inhibitors.

**anti-angiogenic agents, epidermal growth factor receptor inhibitors, and mammalian target of rapamycin inhibitors**

Targeted agents used for TNBC treatment inhibit the function of key signalling pathways that regulate tumour microenvironment, growth, survival, and metastasis. These pathways include vascular endothelial growth factor (VEGF)-mediated angiogenesis, epidermal growth factor receptor (EGFR)-mediated differentiation, and mammalian target of rapamycin (mTOR)-mediated proliferation.

**angiogenesis inhibitors**

Intratumoural expression of VEGF is significantly higher in TNBC than in non-TNBC [64], providing a biological rationale for targeting this pathway in the treatment of TNBC patients. Three first-line metastatic trials have studied the addition of bevacizumab, a monoclonal antibody specific for VEGF, to chemotherapy. In retrospective TNBC subgroup analysis, the E2100 study suggested the addition of bevacizumab to paclitaxel reduced the risk of progression in first-line TNBC patients by 51% and doubled the median PFS (5.3 versus 10.6 months; Table 2) [65]. A similar reduction in the risk of progression in TNBC patient subsets was reported after combining bevacizumab with docetaxel in the Avastin and Docetaxel (AVADO) trial (47%) [65], although no clear benefit for TNBC patients was apparent in the Regimens in Bevacizumab for Breast Oncology (RibBOn)-1 trial, which added bevacizumab to chemotherapy [65]. A meta-analysis of TNBC subgroup data from these first-line phase III trials (N = 621) revealed a 35% reduction in the risk of progression [HR = 0.65 (95% CI 0.538–0.873)] and a net benefit in median PFS of 2.7 months (P < 0.0001) when bevacizumab was added to chemotherapy regimens, compared with chemotherapy alone [72]. Similar improvements were observed in the second-line setting. TNBC subgroup analysis (N = 159) of the phase III RibBOn-2 trial [66] demonstrated a 51% reduced risk of progression and a doubling of median PFS among patients treated with the bevacizumab combination, compared with chemotherapy alone [2.7 versus 6.0 months; HR = 0.49 (95% CI 0.33–0.74), P = 0.0006], along with a trend towards improved survival [median, 17.9 versus 12.6 months; HR = 0.624 (95% CI 0.39–1.007), P = 0.0534]. Risks of bevacizumab in the advanced setting include a possible four-fold increased rate of congestive heart failure [73], as well as increased hypertension, proteinuria, neurotoxicity, febrile neutropenia, and bleeding [74]. The Oncology Drugs Advisory Committee recently rejected Genentech’s appeal not to withdraw the accelerated approval of bevacizumab for BC, based in part on the modest risk-benefit ratio of this agent in the overall population [75].
<table>
<thead>
<tr>
<th>Trial</th>
<th>Line of treatment</th>
<th>Treatment algorithms</th>
<th>TNBC patients (N)</th>
<th>Median age, years (range)</th>
<th>ORR (%)</th>
<th>Median PFS (months); HR [95% CI]</th>
<th>Median OS (months); HR [95% CI]</th>
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<tr>
<td><strong>Anti-angiogenic agents</strong></td>
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<tr>
<td>O’Shaugnessy [65], Rd phase III—subgroup comparison</td>
<td>E2100, first-line</td>
<td>Paclitaxel 90 mg/m² d1, 8, 15 q4w; bevacizumab 10 mg/kg d1, 15 q4w</td>
<td>122</td>
<td>NR</td>
<td>NR</td>
<td>10.6; 0.49 [0.34–0.70]</td>
<td>NR</td>
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<tr>
<td></td>
<td>AVADO, first-line</td>
<td>Docetaxel 100 mg/m² q3w; bevacizumab 15 mg/kg q3w</td>
<td>58</td>
<td>NR</td>
<td>NR</td>
<td>8.2; 0.53 [0.34–0.84]</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Docetaxel 100 mg/m² q3w; bevacizumab 7.5 mg/kg q3w</td>
<td>53</td>
<td>NR</td>
<td>NR</td>
<td>6.2; 0.69 [0.44–1.08]</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>RiBBoN-1 (Tax/Anthra), first line</td>
<td>Docetaxel 100 mg/m² q3w; placebo q3w</td>
<td>52</td>
<td>NR</td>
<td>NR</td>
<td>5.4</td>
<td>NR</td>
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<tr>
<td></td>
<td></td>
<td>Taxane- or anthracycline-based chemotherapy; bevacizumab 15 mg/kg q3w</td>
<td>96</td>
<td>NR</td>
<td>NR</td>
<td>6.5; 0.78 [0.53–1.15]</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taxane- or anthracycline-based chemotherapy; placebo q3w</td>
<td>46</td>
<td>NR</td>
<td>NR</td>
<td>6.2</td>
<td>NR</td>
</tr>
<tr>
<td>Brufsky [66], RiBBoN-2, Rd phase III—subgroup</td>
<td>Second-line</td>
<td>Capecitabine-based chemotherapy; bevacizumab 15 mg/kg q3w</td>
<td>87</td>
<td>NR</td>
<td>NR</td>
<td>6.1; 0.72 [0.49–1.06]</td>
<td>NR</td>
</tr>
<tr>
<td>Curigliano [67], Rd phase II</td>
<td>Second-line+ (≥ 1 prior regimen)</td>
<td>Sunitinib 37.5 mg continuous daily dosing</td>
<td>113</td>
<td>52 (NR)</td>
<td>9</td>
<td>1.7</td>
<td>2.5; 1.16 [0.87–1.55]</td>
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<tr>
<td></td>
<td></td>
<td>Standard of care chemotherapy</td>
<td>104</td>
<td>52 (NR)</td>
<td>12 (P = 0.814)</td>
<td>2.5; 1.16 [0.87–1.55]</td>
<td>(P = 0.847)</td>
</tr>
<tr>
<td>Baselga/Gomez [68, 69], SOLTI-0701, Rd phase II—subgroup</td>
<td>First- or second-line (0–1 prior regimens)</td>
<td>Sorafenib 400 mg BID; capecitabine 1000 mg/m² BID d1–14, q3w</td>
<td>20</td>
<td>NR</td>
<td>NR</td>
<td>4.3; 0.596 [0.3–1.1]</td>
<td>17.5; 0.98 [0.50–1.89]</td>
</tr>
<tr>
<td>Hudis [70], AC01B07, Rd phase II—subgroup</td>
<td>First- or second-line (0–1 prior regimens)</td>
<td>Sorafenib 400 mg BID; gemcitabine 1000 mg/m² d1,8, q3w OR capecitabine 1000 mg/m² BID d1–14, q3w</td>
<td>NR</td>
<td>3.1; 0.57 [0.30–1.09]</td>
<td>NR</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Placebo; gemcitabine 1000 mg/m² d1, 8, q3w OR capecitabine 1000 mg/m² BID d1–14, q3w</td>
<td>27</td>
<td>NR</td>
<td>NR</td>
<td>2.6</td>
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<td><strong>EGFR inhibitors</strong></td>
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<tr>
<td>Baselga [43], BALI-1, Rd phase II</td>
<td>First- or second-line (0–1 prior regimens)</td>
<td>Cisplatin 75 mg/m² d1, q3w x 6; cetuximab 400 mg/m² initial dose; then 250 mg/m² qw</td>
<td>115</td>
<td>NR</td>
<td>20 (P = 0.11)</td>
<td>3.7; 0.67 [0.47–0.97]</td>
<td>12.9; 0.82 [0.56–1.20]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cisplatin 75 mg/m² d1, q3w x 6</td>
<td>58</td>
<td>NR</td>
<td>10</td>
<td>1.5</td>
<td>9.4</td>
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<table>
<thead>
<tr>
<th>Trial</th>
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<th>TNBC patients (N)</th>
<th>Median age, years (range)</th>
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<th>Median OS (months); HR [95% CI]</th>
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<tr>
<td>O’Shaughnessy [45], USOR 04-070, Rd phase II—subgroup</td>
<td>First- or second-line</td>
<td>Irinotecan 100 mg/m² d1, 8, q3w; carboplatin AUC 2.5 d1, 8, q3w; cetuximab 400 mg/m² initial dose; then 250 mg/m² qw</td>
<td>42; 39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NR&lt;sup&gt;g&lt;/sup&gt;</td>
<td>49</td>
<td>4.7 (3.8–5.6)</td>
<td>15.5 [10.4–19.2]</td>
</tr>
<tr>
<td>Carey [36], TBCRC 001, Rd phase II</td>
<td>First-line (0–2 prior regimens)</td>
<td>Cetuximab 400 mg/m² initial dose; then 250 mg/m² qw; carboplatin AUC 2 qw (3 of 4 wks)</td>
<td>71</td>
<td>SI (NR)</td>
<td>17</td>
<td>2&lt;sup&gt;f&lt;/sup&gt; (entire cohort)</td>
<td>12&lt;sup&gt;f&lt;/sup&gt; (entire cohort)</td>
</tr>
<tr>
<td>Finn [71], EGF30001, phase III—subgroup</td>
<td>First-line</td>
<td>Paclitaxel 175 mg/m², d1, q3w; lapatinib 1500 mg daily</td>
<td>71</td>
<td>NR</td>
<td>NR</td>
<td>4.6 (EFS)</td>
<td>NR</td>
</tr>
</tbody>
</table>

<sup>a</sup>Relative to placebo.
<sup>b</sup>15 mg/kg q3w or 10 mg/kg q2w, depending on chemotherapy regimen.
<sup>c</sup>Treatment continued until disease progression.
<sup>d</sup>One patient was ER-positive.
<sup>e</sup>Crossover to cetuximab + carboplatin after progressive disease.
<sup>f</sup>Prespecified subgroup analysis;
<sup>g</sup>Median age for ITT population is 55.1 (29–79) years, N = 115.
<sup>h</sup>Median age for ITT population is 55.4 (30–80) years, N = 114.
<sup>i</sup>The 95% CI values estimated from plot.
<sup>j</sup>Median age for ITT population is 53.5 (32–77) years, N = 81.
<sup>k</sup>Median age for ITT population is 54.2 (30–82) years, N = 79.
<sup>l</sup>Crossover allowed upon progressive disease, cetuximab + cisplatin if progressive disease during the first six cycles; cetuximab alone if progressive disease after the first six cycles.
<sup>m</sup>Cetuximab alone at progression.
<sup>n</sup>Enrolled.
<sup>o</sup>Evaluable for efficacy.
<sup>p</sup>Median age for ITT population is 55 years, N = 79.
<sup>q</sup>Median age for ITT population is 53 years, N = 75.
<sup>r</sup>Crossover to cetuximab + carboplatin after progressive disease.
<sup>s</sup>Approximated value reported.
<sup>t</sup>Twenty-four of these patients crossed over to the cetuximab + carboplatin arm.

AUC, area under the curve; AVADO, Avastin and Docetaxel; BID, bi-daily; Cape, capecitabine cohort; d, day; EFS, event-free survival; HR, hazard ratio; ITT, intention to treat; N, number of patients; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; qxw, every x weeks; Rd, randomised; RiBBOn-1/2, Regimens in Bevacizumab for Breast Oncology-1/2; Tax/Anthra, taxane/anthracycline treatment cohort; TNBC, triple-negative breast cancer; wks, weeks.
Two clinical trials evaluated bevacizumab in the neoadjuvant BC setting and included outcomes for TNBC subgroups. Both GeparQuinto and National Surgical Adjuvant Breast and Bowel Project 40 (NSABP)-B40 combined bevacizumab with neoadjuvant anthracycline-taxane chemotherapy in HER2-negative patients and demonstrated overall improvements in pCR rates for patients receiving bevacizumab compared with chemotherapy alone (GeparQuinto, 18.4% versus 14.9%, \( P = 0.04 \); NSABP-40, 34.5% versus 28.4%, \( P = 0.027 \)) [76, 77]. A prespecified subgroup analysis of TNBC patients (\( N = 663 \)) in the GeparQuinto trial revealed a statistically significant improvement in pCR rates (39.3% versus 27.9%, \( P = 0.003 \)) for patients receiving bevacizumab compared with chemotherapy (supplemental Table S2, available at *Annals of Oncology* online) [76]. In contrast, the NSABP-B40 study demonstrated that adding bevacizumab to chemotherapy did not improve pCR rates in the TNBC subgroup (\( N = 479; 51.3\% \text{ versus } 47.3\%, \( P = 0.44 \)) [77]. As long-term DFS and OS results are not yet available for these studies, the relationships between pCR and standard outcomes in this setting remain speculative. The most critical safety concerns associated with bevacizumab in early-stage disease are hypertension [77] and the potential for cardiotoxicity; short-term cardiac follow-up of adjuvant bevacizumab-treated patients in phase II feasibility studies are inconclusive [78–81].

Cell surface receptor tyrosine kinases (TKs), including VEGF receptors, are critical to angiogenesis, and the effects of two TKIs on endothelial cell proliferation have been evaluated in TNBC. Initial phase II and subsequent randomised trials have shown limited activity for single-agent sunitinib (Sutent®; Pfizer) (Table 2) [67, 84, 85] and significant toxicity for sunitinib–chemotherapy combinations [86–88], including an increase in on-study deaths [67]. Sorafenib (Nexavar®; Bayer) has demonstrated modest single-agent activity [89, 90]; however, three randomised, double-blind, placebo-controlled phase IIb trials have demonstrated improved overall outcomes for sorafenib–chemotherapy combinations in first- and second-line MBC [68, 70, 91]. A prespecified subgroup analysis of TNBC patients in the SOLTI-0701 (\( N = 53 \)) trial showed an improvement of almost two months in median PFS with the addition of sorafenib to capecitabine (2.5 versus 4.3 months; HR = 0.596 (95% CI 0.3–1.1); Table 2) [69] and a trend towards improved PFS in AC01B07 (\( N = 50 \)) [70]. Sorafenib combinations are associated with higher rates of grade 3/4 toxicities such as hand–foot syndrome, stomatitis, and fatigue [68, 70, 91].

Randomised studies in the neoadjuvant BC setting have generated conflicting results regarding the benefits of adding bevacizumab to chemotherapy in TNBC. The TNBC study populations may not be directly comparable given the potential difference in IHC testing between groups [77, 92] and variability in local IHC testing [29]. The trials also differed in selection and sequencing of companion chemotherapeutics [76, 77]. Furthermore, as long-term DFS and OS results are not yet available for these studies, the relationships between pCR and standard outcomes in this setting remain speculative. Clinical and correlational science results of the ongoing phase III Bevacizumab Adjuvant Therapy in TRIple negative Breast Cancer (BEATRICE) trial, comparing standard adjuvant chemotherapy with or without bevacizumab, will significantly clarify the role of anti-angiogenic therapy in early TNBC (NCT00528567).

**EGFR inhibitors**

EGFR has been implicated as a molecular target for treatment of TNBC based on its frequent IHC expression in TN tumours (27%–57%) [8, 93–95]. There is randomised data on EGFR inhibitors, including the monoclonal antibody cetuximab and the TKIs erlotinib and lapatinib (Tykerb; GlaxoSmithKline), in the management of TNBC. Cetuximab has demonstrated efficacious signals in two prospective studies and one retrospective analysis of randomised phase II trials in advanced TNBC (Table 2). The largest EGFR trial, BALI-1, prospectively evaluated the addition of cetuximab to cisplatin for the treatment of first- and second-line TNBC patients (\( N = 173 \)) [43]. The combination was safe, with minimal increased toxicity in the form of acneiform rashes and significantly increased median PFS compared with cisplatin alone. However, the regimen failed to improve ORR or OS, and PFS gains may have been due to the inferior performance of the non-standard control arm.

The addition of cetuximab to irinotecan and carboplatin in first- and second-line MBC patients in the USOR-04-070 trial [45] resulted in improved response rates among a subset of TNBC patients (\( N = 72; \text{ORR, 30\% versus 49\%} \)). However, no improvements in either PFS or OS were apparent for the TNBC subgroup, and the cetuximab combination resulted in a substantial increase in diarrhoea compared with chemotherapy alone (grade 3/4, 11% versus 25%). A third cetuximab trial added carboplatin to cetuximab in heavily pretreated TNBC patients (\( N = 102 \)) and resulted in an ORR of 17%, and prolonged PFS (2 versus 8 months) was seen in responders compared with the overall trial population [36]. PFS and OS outcomes are pending.

A randomised phase II trial assessed the combination of erlotinib with carboplatin and docetaxel in the neoadjuvant treatment of TNBC patients (\( N = 30 \)) and demonstrated promising activity (pCR; 40%; supplemental Table S2, available at *Annals of Oncology* online) [47] with only minimally increased toxicity. Retrospective data from two randomised phase II trials demonstrated modest activity for gefitinib (Iressa®; AstraZeneca, \( N = 82 \)) in combination with standard neoadjuvant chemotherapy (supplemental Table S2, available at *Annals of Oncology* online), and a lack of activity for lapatinib (\( N = 131 \)) in combination with paclitaxel in advanced TNBC patients (Table 2) [71, 95].

The role of TKIs in TNBC is an area of ongoing investigation, as efficacy signals for EGFR-targeted agents in combination with chemotherapy in TNBC have been inconsistent. Ongoing prospective, randomised EGFR clinical trials, such as those investigating combinations of cetuximab with ixabepilone in both the early (NCT01097642) and advanced (NCT00633464) settings, will better define the role of these agents in TNBC.
mTOR inhibitors

Preclinical studies suggest that upregulated mTOR or aberrant phosphoinositide 3-kinase/Akt pathways confer sensitivity to mTOR inhibitors [97, 98], such as everolimus (Afinitor®; Novartis), and disrupt epithelial-mesenchymal transition expression patterns [1]. Although initial clinical results from studies of everolimus and paclitaxel were encouraging [99, 100], a randomised neoadjuvant phase II study in BLBC/TNBC patients (N = 50) evaluating the addition of everolimus to standard chemotherapy did not significantly improve pCR rates (supplemental Table S2, available at Annals of Oncology online) [101]. Ongoing trials will define the role of everolimus in the neoadjuvant setting (NCT00930930; supplemental Figure S2, available at Annals of Oncology online), as well as evaluate other mTOR inhibitors, such as temsirolimus (Torisel®; Wyeth), and dual mTOR/phosphoinositide 3-kinase inhibitors, such as NVP-BEZ235, in the advanced setting (NCT01272141, NCT01111825, NCT01337765).

discussion

The most dramatic improvements in survival in the treatment of BC have been related to targeted therapy. TNBC trials of targeted therapy as single agents or in combination with chemotherapy, despite a strong biological rationale in many cases, have been less promising, resulting in modest gains in PFS and no gains in OS. Retrospective evaluations of antiangiogenic agents have demonstrated encouraging efficacy signals in TNBC, with a doubling of median PFS in select trials [65, 66, 72, 102]. However, PFS gains remain modest (pooled data from first-line bevacizumab trials, 2.7 months, and second-line bevacizumab, 3.3 months) [66, 72]. Outcomes for EGFR and mTOR inhibitors were unremarkable [43, 101]. Although initial PFS and OS gains for the PARP–chemotherapy combinations were promising (3.6 and 3.5 months) [103], phase III outcomes were disappointing [37].

Patient selection and the heterogeneity of TNBC

A compelling explanation for this lack of activity is the mounting evidence that TNBC is heterogeneous (Figure 1) [5, 104]. In contrast to initial trials of targeted agents conducted in biologically preselected patient populations, TNBC is generally a definition of convenience, characterising a patient population defined by a lack of expression of common therapeutic targets: HER2, estrogen receptor, and progesterone receptor, which does not take into account other differences in TN tumours. Furthering our understanding of TNBC subtypes and underlying signalling pathways will be critical to selecting patient populations that preferentially respond to specific targeted agents. A cluster analysis of the gene expression pattern of TNBC by Lehmann and colleagues revealed six different subtypes defined by common aberrations in signalling pathways, including basal-like 1 and 2 (BL1 and BL2), immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor [1]. TNBC cell line models of the identified subtypes demonstrated increased sensitivity to agents targeting subtype-specific enriched canonical pathways, including the preferential response of BL1 and BL2 subtypes to cisplatin and the enhanced susceptibility of the epithelial–mesenchymal transition subtype to an mTOR inhibitor [1]. Moreover, in the clinical setting, analysis of two randomised trials has shown that BLBC patients lacking PTEN and alpha basic crystallin preferentially respond to cetuximab treatment [105].

The susceptibility of BL1 and BL2 subtypes to DNA damaging agents and the favourable outcomes associated with certain platinum combinations in TNBC [41, 48–51, 106, 107] may suggest an association between BRCA1 expression and BL cancers [12, 108, 109]. BRCA1 mutation carriers often display BL gene expression profiles, and there is increasing evidence that a TN, BLBC subtype develops mainly through a BRCA1-related pathway, resulting in increased genomic instability [110]. Consistent with these findings are those of recent neoadjuvant trials indicating efficacy for single-agent cisplatin in small cohorts of BRCA1 mutation-positive BC patients [111, 112], as well as those identifying BRCA1 expression as a predictor of response to single-agent cisplatin in TNBC patients [106]. If BRCA1ness defines a TNBC subpopulation sensitive to platinum derivatives, then clinical trials, such as the Triple Negative Breast Cancer Trial (TN; NCT00532727), substituting carboplatin for docetaxel in TN and BRCA1/2 mutation-positive metastatic BC patients should improve outcomes.

PARP inhibitors have also demonstrated activity in BRCA1/2 mutant populations [54, 55] and BRCA1/2 mutation has been found to predict response to the PARP inhibitors olaparib [55] and veliparib [56], indicating that BRCA-selected patient groups may be amenable to ongoing PARP development. Ongoing biomarker analysis of the phase III iniparib study may clarify the role of iniparib in patients carrying BRCA1/2 mutations [37]. Additionally, the combination of a platinum-based agent with the PARP inhibitor PF-01367338 is being evaluated after preoperative chemotherapy in patients with TN or BRCA1/2-associated BC (NCT01074970).

Selection of targeted therapies and associated targets

Fundamental to improving therapeutic outcomes is an increased understanding of the mechanisms of action of targeted agents and the underlying pathways they inhibit. PARP inhibitors have traditionally been thought to target PARP 1/2-mediated DNA repair mechanisms [113]. The mechanism of action of iniparib is being questioned, as recent preclinical data suggest that iniparib may have less of an effect on PARP 1 and 2 and may work primarily through inhibition of telomere pathways, implicating PARP 5 and 6 as the primary targets [114]. Furthering our understanding of the mechanisms of action of targeted agents will help refine treatment strategies by providing insights into optimal combinatorial strategies, such as the dual targeting of complimentary or synergistic pathways. Of the randomised trials studied in this review, the only trial to report an OS gain evaluated a cisplatin–metronomic chemotherapy combination (Table 1) [35]. Preclinical studies suggest that metronomic chemotherapy produces anti-angiogenic effects [115], while cisplatin decreases microvessel density and expression of VEGF
in preclinical models [116], as well as causes DNA cross-links leading to apoptosis. Thus, the improved outcomes in this trial may have resulted from the inhibition of complimentary or synergistic pathways related to the use of platinum agents and metronomic chemotherapy. Ongoing trials investigating metronomic or dose dense strategies include the adjuvant phase III trial comparing a dose dense anthracycline–taxane regimen to dose dense paclitaxel and carboplatin (NCT01378533), and the neoadjuvant phase II trial evaluating metronomic chemotherapy followed by carboplatin (NCT00542191). Finally, Cancer and Leukemia Group B (CALGB)-40603 is a randomised, phase II study evaluating the combinatorial effects of DNA-damaging and anti-angiogenic agents in the neoadjuvant setting (NCT00861705).

predictors of response to targeted therapy

The development of many targeted therapies has been hindered by an inability to define a patient group that would preferentially benefit from the targeted agent. VEGF expression in bevacizumab-treated patients has been linked with improved survival [117] and, more recently, retrospective analyses have identified delta-like ligand 4, VEGF-C, and neuropilin-1 as predictors of bevacizumab response [118]. Likewise, differences in baseline PARP expression levels may be predictive of response to PARP inhibitors, and phosphatase and tensin homolog genotype may predict activity of PARP inhibitors [119]. Continued investigation into predictive markers is a recommended part of ongoing trials of targeted agents.

clinical trial design and ongoing clinical trials

Critical to the ongoing development of targeted therapy in BC is the need to limit trial enrolment to target-selected populations, and to confirm retrospective or randomised phase II findings in a phase III context. There are currently 22 randomised clinical trials underway in TNBC, six planned phase III trials, and only two trials planned in target-selected patient populations, suggesting that the utility of targeted therapy in TNBC will be uncertain for some time to come.

conclusions

TNBC is a heterogeneous disease characterised by a lack of both HER2 and specific hormone receptor expression. The design of clinical trials based on a strong biological rationale, a focus on biologically preselected populations, the identification of predictors of response, and validation in phase III trials will be essential to the ongoing development and successful future use of targeted therapies in TNBC.

acknowledgements

The authors thank Loretta Collins, Ilidio Martins, and Deborah Card from Kaleidoscope Strategic for their editorial assistance. We also thank Sanofi-Aventis for providing the unrestricted educational grant to fund this initiative. Disclaimer: The views expressed in this article reflect the opinions of the authors and do not necessarily reflect those of the sponsor (Sanofi-Aventis) or of any other private or public entity.

funding

Funding for this project was provided through an unrestricted educational grant from Sanofi-Aventis. The paper was conceived and developed by the authors with the support of an independent clinical research organisation, Kaleidoscope Strategic. This organisation worked directly with the authors to develop content and had no interaction with the sponsor in this matter. The sponsor had no role in review design; collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to submit the manuscript for publication. The role of the sponsor in funding the project is acknowledged in the manuscript. The authors have had full access to study data and have agreed to submit this review for publication.

disclosures

No author has provided expert testimony to, holds stock from, or holds employment or leadership positions in commercial companies concerned with the research, development, marketing, and selling of pharmaceutical products and services. KG has served as a consultant to Roche, Novartis, AstraZeneca, Pfizer, and Amgen; received honoraria from Roche and GSK; and research funding from Roche. RD has served as a consultant to Roche, GSK, Novartis, AstraZeneca, and Sanofi-Aventis; and received honoraria from Roche, GSK, Novartis, AstraZeneca, sanofi-aventis, and Eisai. JRM has served as a consultant to Roche/Genentech, Amgen, Novartis, and Pfizer and received honoraria from Amgen and Roche. KL has served as a consultant to Amgen, sanofi-aventis, and Roche and received honoraria from Amgen, sanofi-aventis, and Roche. DM’s institution has received unrestricted educational grants from sanofi-aventis and Roche; SV has served as a consultant to Novartis, Amgen, Roche, and sanofi-aventis; received honoraria from Amgen, Novartis, Roche, and sanofi-aventis; and research funding from Roche and sanofi-aventis.

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