Triple-negative breast cancer: are we making headway at least?

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Ther Adv Med Oncol
(2012) 4(4) 195–210
DOI: 10.1177/1758834012444711

Abstract: The so-called triple-negative breast cancer, as defined by tumors that lack estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2) overexpression, has generated growing interest in recent years despite representing less than 20% of all breast cancers. These tumors constitute an important clinical challenge, as they do not respond to endocrine treatment and other targeted therapies. As a group they harbor an aggressive clinical phenotype with early development of visceral metastases and a poor long-term prognosis. While chemotherapy remains effective in triple-negative disease, research continues to further identify potential new targets based on phenotypical and molecular characteristics of these tumors. In this respect, the presence of a higher expression of different biomarkers including epidermal growth factor receptor, vascular endothelial growth factor receptor, fibroblast growth factor receptor and Akt activation has led to a proliferation of clinical trials assessing the role of inhibitors to these pathways in triple-negative tumors. Moreover, the described overlap between triple-negative and basal-like tumors, and the similarities with tumors arising in the *BRCA1* mutation carriers has offered potential therapeutic avenues for patients with these cancers including poly (ADP-ribose) polymerase inhibitors and a focus on a higher sensitivity to alkylating chemotherapy agents. Results from these trials have shown some benefit in small subgroups of patients, even in single-agent therapy, which reflects the heterogeneity of triple-negative breast cancer and highlights the need for a further subclassification of these types of tumors for better prognosis identification and treatment individualization.

Keywords: basal-like, BRCA1, breast cancer, PARP, poly(ADP-ribose) polymerase inhibitors, triple negative

Introduction

Triple-negative breast cancer (TNBC) is phenotypically characterized by a lack of expression of estrogen receptor (ER), progesterone receptor (PgR) and the absence of human epidermal growth factor receptor 2 (HER2) overexpression and/or amplification [Dent *et al.* 2007]. This specific group accounts for approximately 15–20% of all breast cancer (BC) types [Bauer *et al.* 2007].

TNBC also typically expresses epidermal growth factor receptor (EGFR) and basal cytokeratins (particularly cytokeratin 5, 14 and 17) [Cheang *et al.* 2008; Viale *et al.* 2009]. In addition, TNBC is frequently associated with high expression of proliferation markers (i.e. Ki67) [Viale *et al.* 2009], high levels of cyclin E, low levels of cyclin D1 [Bostrom *et al.* 2009; Voduc *et al.* 2008] and activation of the beta-catenin pathway [Geyer *et al.* 2011]. Moreover, >50% of TNBC show P53 nuclear expression [Rakha *et al.* 2007].

Although classically considered as synonymous of the basal-like breast cancer (BLBC) molecular subtype described by Perou and colleagues [Perou *et al.* 2000], only 70% of TNBCs present with basal-like molecular characteristics according to gene-expression profiling, so TNBC and BLBC should be regarded as distinct but overlapping categories [Rakha and Ellis, 2009; Tan *et al.* 2008]. In this review, we mainly refer to TNBC, except otherwise specified.

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Epidemiologically, TNBC occurs more frequently in younger patients (<50 years old) and generally harbor a more aggressive behavior [Bauer *et al.* 2007].

In a cohort of 1061 patients with breast cancer, Dent and colleagues [Dent *et al.* 2007] showed an increased risk of distant recurrence following diagnosis among patients with TNBC tumors compared with other subtypes (hazard ratio [HR] = 2.6; 95% confidence interval [CI], 2.0–3.5; $p < 0.0001$). The median overall survival (OS) among patients with TNBC was also shorter than that with other subtypes (4.2 *versus* 6.0 years; $p < 0.0001$). The pattern of distant recurrence was also significantly different between the two groups with a peak of recurrence for triple-negative tumors 1–3 years after the initial diagnosis with a quick drop thereafter. Similar findings were reported in an MD Anderson Cancer Center cohort of more than 1110 patients, the 3-year OS was significantly lower for patients with TNBC than for patients with other subtypes (74 *versus* 89%; $p < 0.0001$) [Liedtke *et al.* 2008]. Finally, TNBCs tend to relapse with distant metastases rather than local recurrences and are more likely to develop visceral metastases including central nervous involvement [Lin *et al.* 2008].

Taken together, data strongly suggest that TNBC has a particularly poor prognosis, usually presenting with high-grade tumors, a short disease-free interval after surgery and adjuvant treatment and a propensity for visceral metastases [Dent *et al.* 2009].

It is noteworthy that TNBC is a heterogeneous disease that includes several subsets of tumors with different prognosis like, for example, the adenoid cystic and secretory carcinomas with unexpectedly good outcome [Azoulay *et al.* 2005; Lae *et al.* 2009; Marchio *et al.* 2010]. On the other hand, the recently identified claudin-low subtype, characterized by low expression of the claudin genes and often presenting with an intense immune cell infiltrate and stem cell features, and features of epithelial-mesenchymal transition, is associated with worse prognosis [Perou, 2011].

Gene expression array studies [Lehmann *et al.* 2011] have identified up to six different TNBC subtypes displaying distinct ontologies including two basal-like, an immunomodulatory, a mesenchymal, a mesenchymal stem-like and a luminal androgen receptor subtype.

Treatment

The overall poor prognosis of patients with TNBC and their tendency to relapse with distant metastases make the need for effective systemic therapies an absolute clinical imperative, especially in the early setting.

Unlike patients with ER/PgR+ and/or HER2-overexpressing disease, systemic treatment options for TNBC are limited to cytotoxic chemotherapy, despite its poor long-term outcome, chemotherapy is remarkably effective in this group of patients [Rouzier *et al.* 2005].

Early disease

Neoadjuvant studies in TNBC have shown high pathological response (pCR) rates to anthracyline- and taxane-based chemotherapy regimen. In a neoadjuvant study conducted by Rouzier and colleagues, the basal-like subgroup determined by gene expression profile was associated with an increased likelihood of pCR after neoadjuvant paclitaxel–FAC (fluorouracil, doxorubicin, cyclophosphamide) chemotherapy (45%; 95% CI 24–68) compared with the luminal subgroup (6%; 95% CI 1–21%) [Rouzier *et al.* 2005].

Similarly, in a prospective cohort of 1118 patients treated at the MD Anderson Cancer Center, patients with TNBC phenotype achieved higher pCR rates with neoadjuvant chemotherapy (mostly anthracycline-based) compared with non-TNBC (22% *versus* 11%, $p = 0.034$) [Liedtke *et al.* 2008].

High clinical response rates were also seen with anthracycline-based regimens in patients with TNBC in other studies [Byrski *et al.* 2010; Carey *et al.* 2007] including the GeparTrio study were triple-negative status was associated with higher pCR rates to TAC (docetaxel, doxorubicin, cyclophosphamide) chemotherapy (38.9% *versus* 15.2%; TNBC *versus* non-TNBC, $p < 0.0001$) [Huober *et al.* 2010].

Despite initial reports that TNBC might not benefit from taxanes, the addition of paclitaxel to AC (doxorubicin, cyclophosphamide) was associated with increased disease-free survival (DFS) rates ($p = 0.002$) in patients with TNBC [Hayes *et al.* 2007]. This benefit has also been confirmed by other groups both in node-positive and node-negative TNBC patients [Hugh *et al.* 2009; Martin *et al.* 2010; Roche *et al.* 2006].

The addition of other chemotherapeutic agents, such as capecitabine to standard chemotherapy in early breast cancer has been evaluated. The FinXX trial presented at San Antonio Breast Cancer Symposium 2010 assessed the benefit of the addition of capecitabine to standard neoadjuvant chemotherapy with three cycles of docetaxel followed by three cycles of EC (epirubicin-docetaxel). No differences were observed in the overall population. Nevertheless, in the subgroup of patients with TNBC (n = 202), there was a increased relapse-free survival (RFS; HR = 0.48; 95% CI 0.26–0.88; p = 0.018) for patients in the capecitabine group [Joensuu *et al.* 2010]. Toxicity for all patients was quite similar between the two groups with higher incidence of grade 3/4 hand–foot syndrome (9.6% *versus* 0%) and diarrhea (6.3% *versus* 3.1%) for patients receiving capecitabine. On the other hand, grade 3/4 infection and neutropenia were seen in the control arm, especially in relation to docetaxel since dose of docetaxel was higher in the control arm [Joensuu *et al.* 2007]. In a similar study, O'Shaughnessy reported a trend for benefit with the addition of capecitabine to docetaxel after adjuvant AC in TNBC patients (HR = 0.64; 95% CI 0.44–0.95) [O'Shaughnessy *et al.* 2010].

Overall, TNBC derives substantial benefit from adjuvant chemotherapy. Nevertheless, a subset of patients will present an early, aggressive metastatic relapse.

Treatment of metastatic/relapsed TNBC: do the current treatments provide clinically meaningful benefit?

Neoadjuvant studies in breast cancer patients have shown evidence that the achievement of pCR to neoadjuvant chemotherapy is associated with good long-term prognosis [Scholl *et al.* 1995]. This becomes more manifest in patients with TNBC since the presence of residual disease after completion of primary systemic treatment is associated with an early risk of relapse with a peak of metastatic event occurring at around 1 year [Liedtke *et al.* 2008].

Although a variety of single agents and combination regimens are available, none is recommended specifically for TNBC. Yet most patients with stage IV disease relapse shortly after (neo)adjuvant chemotherapy with visceral metastases and a short life expectancy [Liedtke *et al.* 2008].

Capecitabine is currently a standard treatment option for metastatic breast cancer patients after anthracycline and taxanes. Different series assessing its benefit as single-agent first-line therapy have shown response rates around 30–50% with described median time to progression of 3.0–4.9 months [Gelmon *et al.* 2006]. While this may hold true for patients with hormone receptor (HR) expression, in TNBC there is the suggestion that capecitabine monotherapy may be suboptimal. In fact, a study by Rugo and colleagues found that capecitabine monotherapy was associated only with a relative risk (RR) of 15% and a PFS of 1.7 months [Rugo *et al.* 2008].

The addition of ixabepilone (an epothilone analog that inhibits microtubule function) to capecitabine doubled RR and increased PFS to 4.2 months. This study was included in a review of five phase II and two phase III trials assessing the combination of ixabepilone and capecitabine in this subgroup of tumors. All studies found a benefit in terms of increased median PFS with the incorporation of ixabepilone [Perez *et al.* 2010].

DNA alkylating agents

Since approximately 70% of the breast cancers with BRCA1 mutation are triple-negative, BRCA1-associated tumors and sporadic TNBCs may share many histopathological features including genomic instability and DNA repair effects [Turner *et al.* 2004]. Based on this deficiency in the in the DNA repair machinery, it has been hypothesized that DNA alkylating agents could be specifically effective in this subset of patients.

A recent study in the preoperative setting identified p53-mutant ER-negative tumors as those most sensitive to high-dose alkylating agents with high levels of pCR in triple-negative tumors treated with dose-intense cyclophosphamide [Lehmann-Che *et al.* 2010]. Falo and colleagues [Falo *et al.* 2007] recently published a trial in a series of operable breast cancer patients treated with primary CMF chemotherapy. In this series of 300 patients, the highest response rate was seen in the group of patients with TNBC.

Furthermore, tissue blocks from patients of MA5 trial were recently analyzed for ER, PgR, HER2, Ki67, CK5/6 and EGFR and for tissue microarray to determine the biological subtype [Cheang *et al.* 2009].

The results showed that in the CEF (cyclophosphamide, epirubicin, and fluorouracil) arm, patients with core basal tumors had a HR of 1.8 (log-rank, $p = 0.02$) for OS relative to the other biological subtypes. In the CMF arm, there was no significant difference (HR = 0.9, $p = 0.7$). The interaction between core basal status and treatment was borderline significant ($p = 0.06$). RFS differences did not reach significance. The authors concluded that data from this randomized trial support the hypothesis that anthracycline-containing adjuvant chemotherapy regimens could be inferior to adjuvant CMF in women with BLBC.

A retrospective analysis of 687 patients with TNBC, diagnosed and treated between January 1995 and December 2008, was performed in order to explore factors that predict for relapse. CMF-containing adjuvant chemotherapy significantly decreased recurrence compared with the anthracycline- or taxane-based regimens (RR = 0.66, 95%; CI 0.45–0.96; $p = 0.030$) [Wang *et al.* 2011].

These results support previous reports in which early breast cancer patients with negative hormone receptors have less or even no survival advantage with anthracycline-based adjuvant chemotherapy compared with nonanthracycline regimens [Gennari *et al.* 2008; Tan *et al.* 2008].

In one retrospective study that examined the pCR rates achieved with different types of neoadjuvant chemotherapy in 102 women with breast cancer carrying the BRCA1 mutation, of the 12 patients treated with single-agent cisplatin, 10 (83%) achieved a pCR [Byrski *et al.* 2010] compared with <25% of the 90 patients treated with other regimens. Sirohi and colleagues studied patients treated with platinum-based chemotherapy in the neoadjuvant, adjuvant and metastatic setting [Sirohi *et al.* 2008]. Although 5-year DFS and OS were worse for TNBC patients compared with other tumor types, neoadjuvant RR were higher (88% *versus* 51%; $p = 0.005$). Response rates were also higher for TNBC patients in the advanced setting (41% *versus* 31%; $p = 0.3$), with improved PFS and a trend for a better OS.

Recently published data from a small prospective study of neoadjuvant single-agent cisplatin in 28 patients with TNBC reported pCR was of 21%, including two patients with BRCA1 germline mutations [Silver *et al.* 2010]. Factors associated with good response to cisplatin included young age ($p = 0.03$) and BRCA1 promoter hypermethylation ($p = 0.04$).

Trabectedin

Trabectedin (ET-743; Pharmamar; Spain) blocks the cell cycle at the G_2 phase. It also inhibits overexpression of the multidrug resistance-1 gene (MDR-1). The agent is also thought to interfere with the nucleotide excision repair pathways of cancer cells, suggesting that it could be effective in the treatment of many cancer types including melanoma and sarcoma, as well as lung, breast, ovarian, endometrial and prostate cancers [Adis R&D Profile, 2006].

As single-agent therapy in breast cancer, trabectedin has been assessed in a large phase II trial given at 1.3 mg/m^2 as a 3-hour iv infusion every three weeks to 55 patients with pretreated progressive metastatic breast cancer. Some activity was seen for patients with HER2-positive tumors (10%) and in BRCA1/2 mutation carriers (14%) [Tedesco *et al.* 2010]. Interestingly, the arm with triple-negative tumors was closed earlier due to lack of efficacy with only two unconfirmed partial responses out of 43 patients (<5%) and a median PFS of 1.5 months (95% CI 1.2–2.9 months).

In a second phase II study evaluating two different dosing regimens of trabedectin in patients previously treated with anthracyclines and taxanes, of the four responses observed from both schedules evaluated, two cases belonged to the triple-negative profile while the other two had HER2 overexpression [Gurtler *et al.* 2005].

New targets

An improved understanding of the biology of TNBC had led to identification of several potential new targets.

As TNBC arises from myoepithelial cells, it shares with these cells the presence of surface biomarkers including CK5-6 and EGFR [Jones *et al.* 2004]. At a molecular level, BLBCs (and, therefore, a substantial part of TNBCs) are characterized by deregulation of different kinases including PTEN losses and an activation of the Akt pathway [Andre *et al.* 2009; Marty *et al.* 2008]. Also observed are the following: fibroblast growth factor receptor (FGFR) 2 amplification [Turner *et al.* 2010], vascular endothelial growth factor A (VEGFA) [Andre *et al.* 2009] and androgen receptor (AR)

overexpression [Niemeier *et al.* 2010] and an enrichment in breast cancer stem cells (CD44+/CD24$^{-/low}$) with deregulation of the NOTCH pathway [Park *et al.* 2010].

Some of the current active clinical trials with targeted agents in TNBC are summarized in Table 1.

DNA repair pathways: PARP inhibitors

A favored mechanism for repair of double-strand breaks is homologous recombination, a BRCA1/2-dependent process in which the homologous sequence is used to precisely repair the break. A patient with an inherited BRCA1 or BRCA2 mutation has normal BRCA function, owing to the one functional allele, but in the cancer this

Table 1. Current clinical trials assessing the role of targeted agents in triple-negative breast cancer.

Phase	Compound	Target	Treatment associated	Stage	Status	Trial ID
I / II	AZD2281 (olaparib)	PARP	Paclitaxel	Advanced	Active not recruiting	NCT00707707
II	BSI 201 (iniparib)	PARP	Gemcitabine, carboplatin	Neoadjuvant	Recruiting	NCT00813956
II	Tigatuzumab	TNFR 10B	Abraxane	Advanced	Recruiting	NCT01307891
II	Iniparib	PARP	Irinotecan	Advanced CNS	Recruiting	NCT01173497
I / II	Sunitinib	VEGFR1 VEGFR2	Paclitaxel, carboplatin	Neoadjuvant	Recruiting	NCT00887575
II	P276 00	HIF-1	Gemcitabine, carboplatin	Advanced	Recruiting	NCT01333137
I	RO4929097	NOTCH	Paclitaxel, carboplatin	Neoadjuvant	Recruiting	NCT01238133
II	Entinostat	Deacetylase inhibitor	Anastrozole	LABC	Recruiting	NCT01234532
I / II	Temsirolimub + neratinib	mTOR / EGFR	None	Advanced	Recruiting	NCT01111825
II	Apatinib	TKI VEGFR	None	Advanced	Recruiting	NCT01176669
II	NK-012	Active metabolite of irinotecan	None	LABC/advanced	Recruiting	NCT00951054
III	Bevacizumab	VEGF	Standard adjuvant chemotherapy	Adjuvant	Active not recruiting	NCT00528567
II	Onartuzumab	c-Met	Paclitaxel	Advanced	Recruiting	NCT01186991
II	SAR240550 (iniparib)	PARP	Gemcitabine, carboplatin	Advanced	Active not recruiting	NCT01045304
II	Everolimus + lapatinib	mTOR / EGFR	None	LABC/advanced	Recruiting	NCT01272141
I / II	Cediranib + olaparib	VEGFR2 / PARP	None	Advanced	Recruiting	NCT01116648
IV	Bevacizumab	VEGF	Taxane	Advanced	Recruiting	NCT01094184
I	Seliciclib	Analog purine base	Liposomal doxorubicin	Advanced	Not recruiting	NCT01333423
II	SAR240550 (iniparib)	PARP	Paclitaxel	Neoadjuvant	Active not recruiting	NCT01204125
II	ABT-888 (veliparib)	PARP	Cisplatin, vinorelbine	Advanced	Recruiting	NCT01104259
I / II	Bevacizumab	VEGF	Carboplatin, paclitaxel	Advanced	Recruiting	NCT00691379
II	Erlotinib	EGFR	Standard neoadjuvant chemotherapy	Neoadjuvant	Active not recruiting	NCT00491816

(Continued)

Table 1. (Continued)

Phase	Compound	Target	Treatment associated	Stage	Status	Trial ID
II	Bevacizumab	VEGF	Carboplatin, docetaxel	Neoadjuvant	Recruiting	NCT01208480
II	Panitumumab	EGFR	Gemcitabine, carboplatin	Advanced	Recruiting	NCT00894504
II	Panitumumab	EGFR	Carboplatin, paclitaxel	Advanced/local recurrence	Recruiting	NCT01009983
II	MM-121	Erb B3	Paclitaxel	Neoadjuvant	Recruiting	NCT01421472
I	AZD2281 (olaparib)	PARP	Carboplatin	Advanced	Recruiting	NCT00647062
NA	Azacitine	demethylating agent	None	Neoadjuvant	Recruiting	NCT01292083
II	Bevacizumab	VEGF	Abraxane, carboplatin	Advanced	Recruiting	NCT00479674
II	Bevacizumab + erlotinib	VEGF / EGFR	Paclitaxel	Advanced	Recruiting	NCT00733408
I / II	Panobinostat	HDAC	Letrozole	Advanced	Suspended	NCT01105312
III	BSI 201 (iniparib)	PARP	Gemcitabine, carboplatin	Advanced	Active not recruiting	NCT00938652
II	RO4929097	NOTCH	None	Advanced/ recurrence	Recruiting	NCT01151449
II	Everolimus	mTOR	Cisplatin, paclitaxel	Neoadjuvant	Recruiting	NCT00930930
II	RAD 001	mTOR	Carboplatin	Advanced	Recruiting	NCT01127763
I	Veliparib	PARP	Carboplatin	Advanced/local recurrence	Recruiting	NCT01251874
II	MK 2206	Akt	None	Neoadjuvant	Recruiting	NCT01319539
II	Azacitidine + entinostat	HDAC		LABC/advanced	Recruiting	NCT01349959
Ib / II	RAD 001	mTOR	Cisplatin, paclitaxel	Advanced	Active not recruiting	NCT01031446
II	RAD 001	mTOR	Paclitaxel	Neoadjuvant	Active not recruiting	NCT00499603
II	Bevacizumab	VEGF	Paclitaxel, capecitabine	Advanced	Recruiting	NCT01069796
II	Bevacizumab	VEGF	Gemcitabine, carboplatin	Advanced	Recruiting	NCT01201265
II	Cetuximab	EGFR	Cisplatin	Advanced	Active not recruiting	NCT00463788
II	Bevacizumab	VEGF	Carboplatin, liposomal doxorubicin	Advanced	Recruiting	NCT00608972
II	Sorafenib	TKI	Cisplatin, paclitaxel	Neoadjuvant	Recruiting	NCT01194869
II	Cetuximab	EGFR	Carboplatin	Advanced	Active not recruiting	NCT00420329
II	Foretinib	VEGFR2 MET	None	Advanced/local recurrence	Recruiting	NCT01147484

EGFR, epidermal growth factor; LABC, Locally advanced breast cancer; mTOR, mammalian target of rapamycin; PARP, poly(ADP-ribose) polymerase inhibitors; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.

allele is usually inactivated, rendering the tumor cells selectively deficient in homologous recombination. Poly(adenosine diphosphate-ribose) polymerases (PARPs) are important regulators of the base excision repair pathway, a DNA repair pathway that becomes vital to cells defective in homologous recombination. Therefore, in tumor cells lacking homologous recombination, pharmacological inhibition of PARP may lead to persistent double-strand breaks, inducing cell death [Farmer *et al.* 2005].

The majority of TNBC are of basal-like molecular subtype and they share similarities with BRCA-1 associated breast cancer, including deficiency in DNA-repairing pathways which may render them more susceptible to PARP inhibition [Turner *et al.* 2004].

Several PARP inhibitors are currently under investigation. The PARP inhibitor, iniparib demonstrated activity in phase I studies [Kopetz *et al.* 2008; Mahany *et al.* 2008] and a resulting phase II trial evaluating the benefit of adding iniparib to gemcitabine and carboplatin in 123 patients with metastatic TNBC previously treated with up to two lines of chemotherapy treatment, showed an impressive increased RR from 32% to 52% (p = 0.02), median PFS (3.6 to 5.9 months; p = 0.01) and OS (7.7 to 12.3 months; p = 0.01) with no significant difference in terms of side effects [O'Shaughnessy *et al.* 2011a].

These encouraging results providing the rationale for an ongoing phase III trial with the same treatment combination. Unfortunately early reports suggest that the trial has failed to meet the prespecified criteria for significance for coprimary endpoints of OS and PFS [O'Shaughnessy *et al.* 2011b], although some benefit was observed in the group of patients that received iniparib in the second and third line of treatment.

Olaparib is a potent PARP1 inhibitor. Results from a phase I/II trial were presented last year. Among the 19 patients advanced breast cancer patients enrolled so far, 37% had investigator confirmed partial responses with the combination of olaparib and paclitaxel, but with higher-than-expected incidence of neutropenia [Dent *et al.* 2010]. On the other hand, a second study using the same compound did not observe any objective responses when administered as single agent in 24 patients with advanced TNBC [Gelmon *et al.* 2010]. Tutt and colleagues [Tutt *et al.* 2010] performed a multicenter phase II study assessing the use of olaparib in 18 BRCA1 and nine BRCA2-deficient breast cancer patients pretreated with a median of three lines of chemotherapy. In this study, two different doses were assessed with results favoring the highest dose (400 mg) *versus* lowest dose (100 mg) in terms of RR (41% and 22%, respectively) and PFS (5.7 *versus* 3.8 months, respectively).

A small trial of another oral PARP inhibitor, veliparib, in combination with temozolamide showed very modest activity in unselected breast cancer, but activity was limited to BRCA-mutation carriers [Isakoff *et al.* 2010].

Recently, the development of some of these PARP inhibitors has been cancelled including olaparib and iniparib. Reasons for this decision remain unclear. In the case of iniparib it could be that, despite its initial designation as a PARP1 inhibitor, its true mechanism of action remains unclear but does not seem to be in relation to PARP inhibition.

Despite this, other compounds that inhibit PARP are currently been tested in different clinical trials (Table 1).

DNA-repair pathways: Chk1 and Chk2 inhibitors

The checkpoint proteins 1 and 2 (Chk1 and Chk2) are critical for cell cycle arrest following induction of double strand breaks [Bolderson *et al.* 2009]. There is evidence that inhibition of Chk1 and Chk2 sensitizes tumor cells to DNA damaging agents *in vitro* and *in vivo* [Ashwell *et al.* 2008; Matthews *et al.* 2007; Zabludoff *et al.* 2008].

Several clinical trials using Chk1 and Chk2 in combination with genotoxic agents including gemcitabine, irinotecan, and cisplatin in different types of solid tumors including breast cancer are underway [Ashwell *et al.* 2008; Ashwell and Zabludoff, 2008].

Even though these clinical trials are mainly in phase I and for all types of cancers, some of them will include TNBC patients, so it is possible that some information might become available for this subgroup of patients.

Antiangiogenic treatment

Recently published data have shown that patients with TNBC have high levels of intratumoral VEGF compared with non-TNBC patients [Linderholm *et al.* 2009]. In addition, a higher proportion of TNBC tumors were found to have a gain in the *VEGFA* gene compared with non-TNBC tumors (34% *versus* 6%) [Andre *et al.* 2009]. Taken together, these results suggest that TNBC could present higher sensitivity to antiangiogenic inhibition [Andre *et al.* 2009].

Many antiangiogenic treatments have been introduced or are currently under development for TNBC patients, although majority of the data available is with the monoclonal antibody bevacizumab.

In the E2100 study, bevacizumab was administered as first-line treatment in metastatic breast cancer in combination with paclitaxel. This trial was conducted in unselected patients but included a majority of HER2-negative patients. A subgroup analysis showed that ER-negative and PgR-negative patients had substantially higher probability of PFS with the addition of bevacizumab (11.4 *versus* 6.11 months; HR = 0.51; 95% CI 0.43–0.62) [Miller *et al.* 2007].

Similarly, in the AVADO trial, the subgroup of patients with TNBC benefited more from the addition of bevacizumab to docetaxel [Miles *et al.* 2010]. A third study (RIBBON-2) [Brufsky *et al.* 2009] observed a 10% increase in RR ($p = 0.01$) with the addition of bevacizumab to chemotherapy in the second-line setting and increase in PFS from 5.5 to 7.2 months ($p = 0.77$). In a recently published parallel study (RIBBON-1), the addition of bevacizumab to first-line chemotherapy was not associated with increased benefit in the subgroup of patients with TNBC.

A meta-analysis that included the 621 patients with TNBC from the E2100, AVADO and RIBBON-1 trials was reported at the San Antonio Breast Cancer Symposium [O'Shaughnessy *et al.* 2009]. For patients with TNBC the addition of bevacizumab was associated with a significant improvement in median PFS (8.1 months for the combination *versus* 5.4 months for chemotherapy alone, HR 0.65, $p < 0.001$) and RR (42% combination *versus* 23% chemotherapy alone, $p < 0.0001$), but not OS (median 18.9 months for the combination *versus* 17.5 months for chemotherapy alone, HR 0.96, $p = 0.67$) or 1-year survival (71% combination *versus* 65% chemotherapy alone, $p = 0.11$). Consistent improvement in PFS was seen in all subgroups examined, including those with a short disease-free interval (≤24 *versus* >24 months), multiple metastatic sites (≥3 *versus* <3) and visceral metastases. The safety profile was consistent with that seen in the overall population.

Preliminary data from one phase II study of neoadjuvant cisplatin plus bevacizumab in TNBC found an overall RR of 63% but a modest pCR (defined as Miller–Payne 5) of 15% [Ryan *et al.* 2009].

Based on all of these previous results, there is an ongoing trial investigating bevacizumab in combination with adjuvant chemotherapy in TNBC (BEATRICE [ClinicalTrials.gov identifier: NCT00528567]) and another in HER2-negative tumors (CALGB 40603), as well as phase II trials in triple-negative patients in the neoadjuvant and metastatic settings [Tan and Swain, 2008].

Recently, the results from two clinical trials in the neoadjuvant setting have shown a benefit from the addition to bevacizumab to chemotherapy in patients with locally advanced TNBC [von Minckwitz *et al.* 2012; Bear *et al.* 2012]. In the first, von Minckwitz and colleagues randomized 1948 patients with a median tumor size of 40 mm on palpation to receive neoadjuvant epirubicin and cyclophosphamide (EC) followed by docetaxel (D), with or without concomitant bevacizumab [von Minckwitz *et al.* 2012]. Rates of pCR were higher among the 663 patients with TNBC (27.9% *versus* 39.3%, control *versus* bevacizumab arm, respectively; $p = 0.003$) for only 7.8% and 7.7% among 1262 patients with HR-positive tumors ($p = 1.00$). In the second study, 1206 patients to receive neoadjuvant therapy consisting of docetaxel (100 mg/m^2), docetaxel (75 mg/m^2) plus capecitabine (825 mg/m^2 d1–14), or docetaxel (75 mg/m^2) plus gemcitabine (1000 mg/m^2, d1, d8) for four cycles, with all regimens followed by treatment with doxorubicin–cyclophosphamide (AC) for four cycles [Bear *et al.* 2012]. Patients were also randomly assigned to receive or not to receive bevacizumab (15 mg/kg) for the first six cycles of chemotherapy. Neither the addition of capecitabine nor gemcitabine to docetaxel therapy, as compared with docetaxel therapy alone, significantly increase the rate of pCR. On the other hand, pCR was significantly increased

by the concomitant administration of bevacizumab, especially in the HR-negative subgroup.

More scanty data are available regarding small-molecule angiogenesis inhibitors. One phase II study of sunitinib in 64 pretreated patients (20 with triple-negative tumors), 61 previously treated with anthracyclines and taxanes, reported seven partial responses, three in triple-negative tumors [Burstein *et al.* 2008].

A phase III randomized study evaluated sunitinib *versus* capecitabine in patients with previously treated HER2-negative advanced breast cancer [Barrios *et al.* 2009]. More than 30% of the patients had triple-negative disease and less than two prior regimens for metastatic disease. The primary end point, DFS, was not met and the trial was stopped prematurely.

Two phase IIb trials evaluating efficacy and safety of sorafenib with chemotherapy or placebo have been presented [Baselga *et al.* 2009; Gradishar *et al.* 2009]. The SOLTI-0701 trial evaluated the combination of sorafenib (400 mg twice daily) with capecitabine in patients with metastatic breast carcinoma (first or second line). A total of 30% of patients had triple-negative disease. Improved median PFS was observed in patients treated with the combination of sorafenib–capecitabine in comparison with sorafenib–placebo (HR = 0.57; p = 0.0006). The incidence of grade III hand–foot was 45% *versus* 13% in the placebo group.

A second trial evaluated sorafenib in combination with paclitaxel or placebo, as first-line therapy in patients with locally recurrent or metastatic breast cancer. A total of 40% of patients had triple-negative disease. The HR for PFS was 0.78 (p = 0.08). The incidence of grade III hand–foot syndrome was 30% *versus* 3% in the placebo group, a trend favoring the sorafenib–paclitaxel group [Gradishar *et al.* 2009].

EGFR inhibitors

EGFR overexpression is found in around 45–70% of TNBC [Nielsen *et al.* 2004], although there is no data to support its activation in breast cancer. As a result of this finding, EGFR-targeted therapy is being evaluated in clinical trials in TNBC and/or BLBC.

In the prospective phase II study by the Translational Breast Cancer Research Consortium (TBCRC) group, single-agent cetuximab was evaluated alone or in combination to carboplatin in patients with TNBC. Response rate when combined was 17% [Nielsen *et al.* 2004]. Importantly, the RR of 6% observed with cetuximab alone could suggest that this treatment might be beneficial for a small subgroup of patients. In a subset of patients in the US Oncology 225200 Trial, the addition of cetuximab to carboplatin and irinotecan in 78 TNBC patients led to a higher RR (49% *versus* 30%) that did not translate into an improvement in PFS (5.1 *versus* 4.7 months) [O'Shaughnessy *et al.* 2007]. Recently, another randomized phase II trial showed modest benefit with the addition of cetuximab to cisplatin in TNBC for PFS (HR = 0.675; p = 0.032) and a nonsignificant increase in RR (20% *versus* 10.3%; p = 0.11) [Baselga *et al.* 2010].

PI3K/Akt/mTOR

PTEN losses have been observed in around 30% of TNBC [Andre *et al.* 2009] and these have been found to be associated with activation of Akt in TNBC samples [Marty *et al.* 2008]. There is therefore the rationale for the use of mTOR (mammalian target of rapamycin) inhibitors in patients with TNBC with PTEN loss.

In the preclinical setting, it has been suggested that mTOR activation could be linked to resistance to treatment with cisplatin [Liu *et al.* 2007; Mabuchi *et al.* 2009]. Interestingly, Beuvink and colleagues [Beuvink *et al.* 2005] reported that adding everolimus to cisplatin could increase by fivefold the loss of viability *in vitro*.

An *in vivo* study of nude mice bearing tumor xenografts of the triple-negative MDA-MB-231 breast cancer cells showed that combination treatment with rapamycin and cyclophosphamide achieved a dramatic reduction in the tumor volume by around 95% ($p < 0.001$) with a synergistic effect between the two drugs [Zeng *et al.* 2010].

In the clinical setting, several ongoing clinical trials are planning to assess the role of mTOR inhibitors in TNBC in combination with different agents (see http://www.cancer.gov) (Table 1).

Recent preclinical data have shown a possible synergism between PARP inhibitors and PI3K inhibitors in breast cancer cell lines with or without BRCA1 and/or PTEN treated with this compounds [Kimbung *et al.* 2012]. In this study,

the administration of PARP inhibitors caused DNA damage by conferring G2/M arrest and decreased viability with an increase of apoptosis. PI3K inhibitors alone decreased also cell growth but due to a G1 arrest. More importantly, when administered together, PARP and PI3K inhibitors interacted synergistically to significantly decrease cell growth compared with any of these compounds alone. These preclinical data could open the possibility of clinical trials with this combination in patients with TNBC.

Src inhibitors

The Src tyrosine kinase is often overexpressed in breast cancer, and this is associated with increased invasiveness and metastatic disease progression [Hiscox *et al.* 2006; Verbeek *et al.* 1996].

Preclinical data indicates that BLBC cell lines are particularly sensitive to Scr inhibition [Finn *et al.* 2007], providing the principle for clinical research in this specific subgroup.

However, in a phase II trial, the antitumor activity of the dual Abl/Src kinase inhibitor dasatinib was modest when given as monotherapy to heavily pretreated patients with TNBC [Liu *et al.* 2007].

Further trials of dasatinib and other dual inhibitors (bosutinib and saracatinib) alone or in combination with chemotherapy, are ongoing, although most are in unselected breast cancer.

AR-targeted therapy

The AR, a member of the steroid hormone receptor family, is expressed in more than 70% of breast cancers and has been implicated in breast cancer pathogenesis. The role of the AR is of particular interest in patients with estrogen and progesterone receptor negative and HER2-negative cancers that do not benefit from conventional endocrine-targeted therapies. Emerging evidence suggests that the AR may serve as a therapeutic target for a subset of TNBCs. An unsupervised cluster analysis of 99 primary breast cancer samples and eight breast cancer cell lines identified a subset of ER-negative and PgR-negative tumors with paradoxical expression of genes known to be either direct targets of ER or responsive to estrogen or typically expressed in ER-positive tumors [Doane *et al.* 2006]. These tumors were in fact found to be characterized by AR expression and transcriptionally regulated by androgen.

In a series of 135 breast cancer in women tested, consecutive paraffin sections were examined immunohistochemically for AR, ER, PgR and HER-2/neu. AR was expressed in 30% (13 of 43) of BRCA1-related tumors, with 21% being also ER negative. For BRCA2-related tumors AR was expressed in 78% (14 of 18) and in 76% (56 of 74) of the BRCA1/2-negative tumors [Pristauz *et al.* 2010].

Although the incidence of AR positivity is lower in TNBC, it is important because there are few proven and effective therapies for these patients.

Abiraterone (CB7598), a selective and irreversible inhibitor of CYP17, has proven efficacy in castrated-resistant prostate tumors [Reid *et al.* 2010]. It is currently being tested in an ongoing phase I/II clinical trial in women with advanced breast carcinoma both in ER-positive and ER-negative with AR expression (see http://www.clinicaltrials.gov).

A similar phase II study using in this case the AR inhibitor, bicalutamide, in patients with triple-negative but AR-positive breast cancer is currently underway (see http://www.clinicaltrials.gov).

FGFR inhibitors

Recently, FGFR2 has reported to be amplified in a subgroup of patients with TNBC [Turner *et al.* 2010]. FGFR2 inhibition in cell lines harboring FGFR2 amplification led to decrease in cell proliferation.

Several clinical trials with FGFR inhibitors in patients with TNBC are currently underway, although the low frequency of FGFR2 amplification could undermine their results.

Conclusions

Despite its low frequency, TNBC has been the focus of extensive research during the last years, principally due to its more aggressive behavior with poorer outcome and the fact that they do not respond to endocrine or HER2-targeted therapy. The finding by molecular analysis of high levels of expression of different genes linked to growth and survival pathways like *EGFR, VEGFR* and *FGFR* and increased activation of Akt, led to the set up of different studies targeting these receptors and pathways. Moreover, based on the finding that

some triple-negative and basal-like tumors may harbor a dysfunctional BRCA1, numerous clinical trials and retrospective analysis focusing in the use of alkylating agents with or without the addition of PARP inhibitors were carried out. All together, these studies have shown that each agent provides a small benefit in TNBC suggesting that further disease segregation and subclassification of this type of tumor is needed to identify which patients will derive the highest benefit from each of these targeted agents for a more individualized treatment. In relation to this, there has been increased interest during recent years to move towards a more personalized medicine based on molecular and genetic characteristics, instead of tumor location [Tursz *et al.* 2011]. This has been encouraged by the increased number of targeted therapies and the introduction of high throughput technologies. The landscape in clinical research in oncology (including breast cancer) it is going to be affected with possibly more phase II studies including patients with specific molecular alterations and less large phase III studies with no patient selection [Andre *et al.* 2011]. Moreover, there is growing interest in implementing the use of high-throughput technologies in daily practice in order to identify molecular alterations in patients to drive patients to targeted therapy or to specific clinical trials. This has already proven as feasible in a study performed in our center in a group of 108 patients with advanced breast cancer (including TNBC) [Arnedos *et al.* 2011]. Targetable molecular alterations were identified in 50% of the patients.

This is of crucial importance especially in tumors such as TNBC, where the only standard treatment available is chemotherapy in order to identify possible molecular alterations. As mentioned before, TNBC is a heterogeneous group with possibly several subtypes with different driven molecular alterations [Lehmann *et al.* 2011]. This could explain the small benefit observed in different clinical trials with different types of targeted therapy in TNBC. Therefore identifying the possible driver in a specific tumor becomes crucial in this aggressive tumor.

One of the recently identified subtype of is the more aggressive claudin-low subtype, characterized by low expression of the claudin genes which often presents with an intense immune cell infiltrate and stem cell features and features of epithelial–mesenchymal transition [Perou, 2011]. These characteristics could open the possibility of immunotherapy treatment in these tumors in the same way that immunotherapeutics have proven to be effective in other cancer types also depending on immune infiltrate like ipilimumab treatment in melanoma [Robert *et al.* 2011] and are currently being tested in other types of tumors like non-small cell lung cancer, small cell lung cancer and metastatic hormone-refractory prostate cancer.

With the use of these new technologies and a much better understanding of its biology together with some encouraging preclinical data with novel therapies, there is hope for improving the outcome and evolution of this disease.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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