

Neoadjuvant treatments in triple-negative breast cancer patients: where we are now and where we are going

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Abstract: Triple-negative breast cancer (TNBC) remains the poorest-prognosis breast cancer (BC) subtype. Gene expression profiling has identified at least six different triple-negative subtypes with different biology and sensitivity to therapies. The heterogeneous nature of TN tumors may justify the difficulty in treating this BC subtype. Several targeted agents have been investigated in clinical trials without demonstrating a clear survival benefit. Therefore, systemic chemotherapy remains the cornerstone of current clinical practice. Improving the knowledge of tumor biology is mandatory for patient management. In stages II and III, neoadjuvant systemic treatment is an effective option of care. The achievement of a pathological complete response represents an optimal surrogate for survival outcome as well as a test for tumor drug sensitivity. In this review, we provide a brief description of the main predictive biomarkers for tumor response to systemic treatment. Moreover, we review the treatment strategies investigated for TNBCs in neoadjuvant settings focusing on experimental drugs such as immunotherapy and poly [ADP-ribose] polymerase inhibitors that hold promise in the treatment of this aggressive disease. Therefore, the management of TNBC represents an urgent, current, unmet need in daily clinical practice. A key recommendation is to design biology-driven clinical trials wherein TNBC patients may be treated on the basis of tumor molecular profile.

Keywords: triple-negative breast cancer, neoadjuvant chemotherapy, BRCA, platinum, immunotherapy, PARP-1 inhibitors

Introduction

Currently, the treatment of patients with triple-negative breast cancer (TNBC) is the biggest challenge in the breast cancer (BC) scenario. TNBCs are defined by the absence of both hormone (estrogen and progesterone) receptors and HER2 overexpression.¹ TNBCs represent a heterogeneous group of BCs with different treatment sensitivity and prognosis. Preclinical studies have identified at least six different molecular subtypes: two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR).²

In the early stages, the use of neoadjuvant systemic treatment (NST) is the standard of care in TNBCs. Patients who achieve a pathological complete response (pCR) with primary therapy have improved survival outcomes.^{3,4} Standard neoadjuvant regimens include anthracyclines, taxanes, and cyclophosphamide.^{5,6} Platinum-based chemotherapy has been proposed but is not yet recommended by available guidelines. Different systemic treatment options have been investigated besides the use of chemotherapy.⁷⁻¹² However, there are no approved targeted therapies for TNBC in the neoadjuvant setting,

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although many different drugs have been studied and still others are currently being tested.

Clearly, there is a major need to better understand the characteristics and the clinical behavior of TNBCs with an aim to develop effective treatments for this BC subtype. The identification of molecular targets is essential for the design of clinical trials that investigate new treatment strategies. In this article, we review the literature on the use of NST in TNBCs. We focus on the molecular markers able to predict response/resistance to therapies. Moreover, we review the recent data on experimental drugs tested, and discuss findings concerning immunologic checkpoint inhibitors in this population. The main, ongoing clinical trials conducted in this field are reported as well.

Predictive factors of response/resistance to neoadjuvant treatment

The known, investigated, predictive factors of response/resistance to NST in patients with TNBC are shown in Figure 1 and listed further.

Breast-related cancer antigen 1

TNBCs are likely to be breast-related cancer antigen 1 (*BRCA1*) mutation carriers or to have gene expression profiles similar to *BRCA1*-deficient tumors.¹³ *BRCA* genes play an important role in DNA double-strand break repair,

contributing to the maintenance of DNA stability.¹⁴ Approximately 20% of all TNBCs show loss or inactivation of *BRCA* genes, resulting in an inefficient repair mechanism.^{15,16} Besides a mutation in *BRCA1/BRCA2*, hypermethylation of both – the *BRCA1* promoter and Fanconi anemia gene (*FANCF*) – result in a *BRCA*-like phenotype (also called the “*BRCAness*” tumors). The *BRCA* status is considered a predictive factor of response to chemotherapy and poly [ADP-ribose] polymerase (PARP) inhibitory agents. With regard to chemotherapy, tumor cell lines lacking functional *BRCA1* or *2* have increased sensitivity to DNA cross-linking agents such as platinum and to DNA-damaging chemotherapy agents such as anthracycline (Table 1).¹⁷ In two clinical studies conducted in TNBC patients treated with neoadjuvant single-agent cisplatin, pCR rates in *BRCA*-mutated women were 100% and 83%, respectively.^{15,18} Moreover, a significant difference in pCR rates has been found between *BRCA1*-mutated and *BRCA1*-wild-type women treated with anthracycline and taxane regimens (57.1% vs 29%; $p < 0.001$).¹⁶ On the other hand, preclinical evidence has shown a negative correlation between the *BRCA1* mutation and taxane sensitivity due to the loss of a pro-apoptotic pathway activated in response to taxane-induced DNA damage.¹⁹ Furthermore, the increased sensitivity to DNA double-strand break agents has been confirmed in *BRCA*-like tumors that have a homologous recombination deficiency (HRD) similar to *BRCA*-mutated

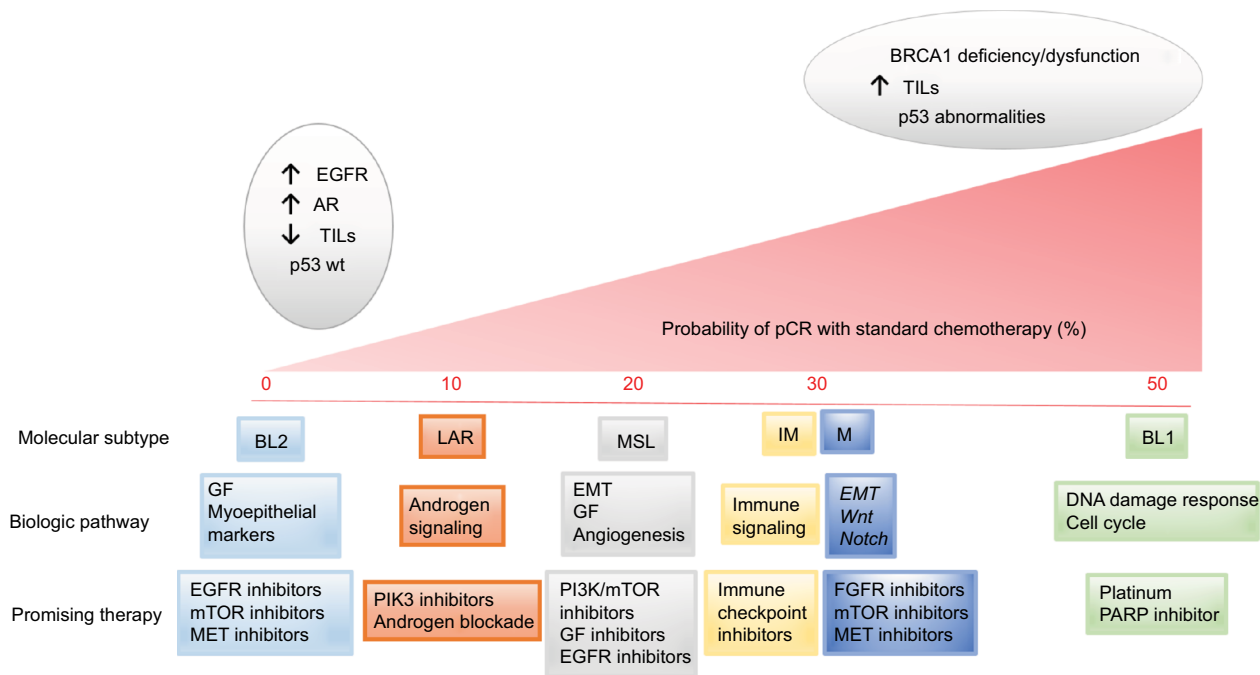


Figure 1 Triple negative breast cancer (TNBC) molecular subtypes classified according to gene expression and the main involved pathways. Each of these subclasses show varying pathological complete response (pCR) rates following standard neoadjuvant chemotherapy. Promising therapies for every molecular subtype have been suggested.

Table 1 pCR rate reported in published clinical trials in TNBC *BRCA*-mutated (*BRCA* mt) patients

| Study | Study design | TNBC n | <i>BRCA</i> mt n | Treatment | pCR definition | pCR <i>BRCA</i> mt % |
|----------------------------|-----------------|-----------|---------------------|--------------------------------------|----------------|-------------------------|
| Byrsky et al ¹⁵ | Phase II | 107 | 82 | Cisplatin | ypT0/is ypN0 | 61 |
| Wang et al ¹⁶ | Retrospective | 956 | 68 | Anthracycline +/- taxane/taxane | ypT0/is ypN0 | 53.8 |
| Silver et al ¹⁸ | Phase II | 28 | 2 | Cisplatin | ypT0/is ypN0 | 100 |
| Telli et al ²⁰ | Pooled analysis | 93 | 19 | Carboplatin + gemcitabine + iniparib | ypT0/is ypN0 | 47 |

Abbreviations: TNBC, triple negative breast cancer; pCR, pathological complete response.

ones. A pooled analysis of six phase II trials conducted in TNBC patients treated with platinum demonstrated that patients with a high HRD score had an increased pCR rate compared to HR-non-deficient patients (53% vs 18%) regardless of the *BRCA* mutation status.²⁰

Molecular subtypes

Lehmann et al analyzed gene expression profiles in 587 TNBCs and identified six different subtypes: BL1, BL2, IM, M, MSL, and LAR.²¹ BL tumors are characterized by a high frequency of chromosomal rearrangements, genomic instability, and *BRCA1* or *BRCA2* mutations. In particular, BL1 tumors are usually enriched in cell-cycle and DNA-damage-response genes that justify their high sensitivity to DNA-damaging agents such as platinum. In contrast, BL2 cancers frequently overexpress growth factor receptors, such as epidermal growth factor receptor (EGFR), IGF1R, and myoepithelial markers with low probability of tumor response to chemotherapy.²¹ More recent data, presented at the San Antonio Breast Cancer Symposium (SABCS) 2016, confirmed how BL1 tumors were likely to achieve a higher pCR rate compared to other TNBC subtypes (38% vs 20%, $p = 0.015$).²² Both M and MSL are enriched in pathways associated with EMT (epithelial–mesenchymal transition) and cell motility. This BC subtype frequently presents PI3KCA-activating mutations. According to genomic expression, mesenchymal tumor cells have displayed responses to dasatinib (abl/src inhibitor) and a PI3K/mTOR inhibitor. The LAR subtype cells express androgen receptors with sensitivity to an AR antagonist such as bicalutamide. Finally, IM tumors are enriched in genes involved in immune cell processes and may be considered the more promising subtypes for immunotherapies.²¹ This molecular classification seems to have not only a predictive value but also a prognostic one. A significant difference in relapse-free survival (RFS) has been found among molecular subtypes. In particular, LAR tumors show a decreased RFS compared with the BL1, IM, and MSL subtypes (HR = 2.9, 3.2, and 10.5, respectively;

$p < 0.05$). There were no reported significant differences in terms of distant-metastasis-free survival (DMFS).²¹

Epidermal growth factor receptor

The EGFR is a transmembrane tyrosine kinase receptor localized on the cell surface that induces cell proliferation, angiogenesis, and apoptosis inhibition.^{23–25} EGFR abnormalities are reported in 27%–57% of TNBCs.²⁶ Clinical data suggested a possible predictive and prognostic value of the EGFR. A retrospective analysis of 117 patients, 28 of whom had a TNBC, showed that EGFR expression was related to a worse response to anthracycline-based NST and poor overall prognosis ($p = 0.03$).²⁷ Moreover, in a multivariate analysis of a retrospective study conducted in 287 women with TNBCs, EGFR overexpression was a significant independent prognostic factor for relapse (31% in EGFR-positive vs 16.2% in EGFR-negative patients).²⁸ Preclinical data showed how the use of anti-EGFR antibodies can decrease antitumor activity by downregulation of EGFR (endocytosis and degradation) and limit cell migration.²⁹ Based on this evidence, a multicenter, prospective, single-arm phase II study was conducted among 60 women with stages II and IIIa TNBC, with an aim to investigate the role of panitumumab (anti-EGFR antibody) in addition to NST (fluorouracil+epirubicin + cyclophosphamide [FEC] followed by docetaxel).¹² The pCR rates were 46.8% in breast and nodes, and 55.3% in the breast only. EGFR expression was confirmed as a predictive factor for response to chemotherapy plus panitumumab. A positive trend for pCR was shown in EGFR overexpressed BC (58% vs 28%, $p = 0.079$), suggesting a potential benefit of anti-EGFR therapy plus chemotherapy in this subgroup of patients.¹²

Tumor-infiltrating lymphocytes

Since the last decade onward, the immune system has been under investigation as a possible target in the management of different cancer subtypes. Tumor-infiltrated lymphocytes (TILs) have been identified in both tumor and stromal tissues. Intratumoral TILs (It-TILs) are lymphocytes that have a direct interaction with cancer cells, whereas stromal TILs (Str-TILs)

are lymphocytes localized in the peripheral stromal area. Considering BC subtypes, TNBCs have the highest tumor TIL expression (~20%) compared to other BC subtypes.^{30,31} In particular, the IM subtype of TNBC is characterized by high presence of immune cells, antigen presentation, and activation of immune pathways.²¹ Clinical data suggested a predictive role of TILs in terms of pCR in patients treated with NST, mainly with platinum regimens.^{32–34} A prospective evaluation of TILs in the GeparSixto trial found that lymphocyte-predominant (LP) BC, defined as tumors with lymphocytic infiltrate greater than 50%, were more likely to achieve pCR as compared to non-LP BCs (59.9% vs 33.8%; $p < 0.001$).³⁵ The addition of carboplatin to anthracycline and taxane in LPBCs further increased the pCR rate up to 75% ($p = 0.002$).³⁵ The predictive role of TILs primarily suggested by some small retrospective data^{36–39} was confirmed by the large meta-analysis present at the SABCs 2016, where a total of 3,771 tumors from the clinical Gepar studies (GeparDuo, GeparTrio, GeparQuattro, GeparQuinto, GeparSixto, and GeparSepto) were evaluated for the presence of TIL.⁴⁰ These results suggest TILs are a strong predictive marker for response to NST in all BC subtypes.⁴⁰ This predictive value was translated into a survival benefit in the TNBCs group. The presence of TILs in residual tumor disease after primary chemotherapy seems to be related to more favorable long-term outcomes as well.⁴⁰ Another retrospective cohort of 278 patients with TNBCs correlated TIL presence with a risk reduction of metastasis and death. The major prognostic relevance of TILs was found in patients with residual tumor >2 cm and/or node metastasis.³³ These authors hypothesized a possible use of TILs for selecting patients with high risk of relapse after NST. A comparison between TIL expression in the BC tissue before and after chemotherapy administration was also undertaken, showing that chemotherapy switches low-TIL tumors into high-TIL tumors.³³ Cytotoxic drugs are able to modify the tumor microenvironment, thus inducing cross-presentation of new peptide antigens, dendritic cell activation, and specific cytotoxic T cells.^{41–46} This evidence supports the idea that the efficacy of immunotherapy may be amplified by chemotherapy.³³ Based on these data, several neoadjuvant trials studying the addition of immunotherapy to chemotherapy in TNBC patients are ongoing.

Focusing on the subsets of TILs, there are two different functional components: cytotoxic CD8+ T cells and regulatory FOXP3+ T cells. CD8+ TILs lead to tumor cell death through linking foreign antigens on tumor cells.^{47–49} In contrast, FOXP3+ TILs, have a critical role in suppressing antitumor immunity. Miyashita et al published a retrospective

multicenter study which evaluated CD8+ TIL, FOXP3+ TIL, and CD8/FOXP3 ratios before and after NST in TNBC tissue.^{50,51} The results showed that patients with high CD8+ TILs had a smaller residual tumor (≤ 2 cm) than patients with low-TILs ($p = 0.005$).⁵² No association between residual tumor size and FOXP3+ TILs or the CD8/FOXP3 ratio was found. Both high CD8+ levels and higher CD8/FOXP3 ratio were associated with improved recurrence-free survival and breast-cancer-specific survival ($p < 0.0001$).⁵² These data have been confirmed in a large meta-analysis, where the absence of both CD8+ TILs and FOXP3+ TILs were associated with worse disease-free survival (DFS) and overall survival (OS).⁵³

PD-1/PD-L1

The programmed cell death protein 1 (PD-1) is expressed on the surface of T cells, and is an immune checkpoint that inhibits T-cell effector function within tissues. PD-1 has two ligands known as PD-L1 and PD-L2; in particular, PD-L1 expression is present in several tumor types. The ligand between PD-L1 and PD-1 on the surface of a lymphocyte blocks the immune response against cancer cells.⁵⁴ The presence of PD-L1 in the tumor microenvironment seems to indicate an adaptive immune resistance to endogenous antitumor activity.⁵⁵ With regard to BC, PD-L1 expression has been found in 50% of all BC subtypes. Its expression was mainly associated with high histological grade and negative hormone receptors.⁵⁶ The first study to investigate PD-L1 expression (defined as cell-surface membrane staining >5%) conducted in BC found a higher PD-L1 expression in TNBCs as compared to non-TNBCs ($p < 0.001$).⁵⁷ In a large study, PD-L1 expression was found to be positive in 64% of the cell membrane, 80% of cytoplasm, and in 93% of tumor stroma in TNBCs. In these cases, PD-L1 expression was related to better survival outcomes.⁵⁸ Despite this evidence, it is well known that PD-L1 is a dynamic marker that changes rapidly over time. The tumor microenvironment, as well as systemic cytotoxic treatment and radiation, influence the immune system thereby determining PD-L1 expression changes. Therefore, a biopsy at one time point may not accurately reflect the real tumor microenvironment. For this reason, tumor PD-L1 expression can be considered to only reflect an immune-active microenvironment with activated T cells in an immunocompetent host, and is not an appropriate predictive biomarker to select patients for immunotherapeutic treatment.⁵⁹ Clinical data on the use of anti-PD-1 or anti-PD-L1 antibodies in different cancer subtypes showed how response is possible even in patients with low PD1/PD-L1 expression.^{60–64}

Androgen receptor

The LAR TNBC is characterized by high androgen receptor (AR) and luminal cytokeratin expression.²¹ Overall, ARs are expressed in 12%–36% of TNBCs.^{65–68} LAR BCs are usually associated with low grade and tumor size, mainly present in postmenopausal women.⁶⁹ Significantly better DFS, RFS, and OS have been reported on comparing LAR to non-LAR TNBCs.^{21,70,71} AR expression seems to be a predictive biomarker for tumor resistance to chemotherapy. When considering neoadjuvant anthracycline/taxane-based chemotherapy, LAR tumors have the lowest pCR rate (0%–10%) as compared to other TNBCs.^{21,70,71} On the other hand, AR expression could be considered a predictive marker for tumor response to antiandrogen therapies. Several preclinical studies demonstrated the sensitivity of the LAR cell line to antiandrogen medication.²¹ Xenograft studies with the use of bicalutamide or enzalutamide support the hypothesis that anti-androgen therapy may be useful for such tumors.^{21,72} In the metastatic setting, clinical evidence suggested the efficacy of these two drugs in terms of clinical benefit rate and progression-free survival (PFS) in patients with AR-positive BC.^{10,11} A phase IIB study (NCT02689427) conducted in AR-positive TNBCs treated with enzalutamide plus paclitaxel in the neoadjuvant setting is currently ongoing.⁷³

Tumor suppressor gene p53

In the subgroups of TNBCs, BL tumors often overexpress genes that codify for proteins involved in cell-cycle and DNA-damage response. p53 is a tumor suppressor protein which plays a key role in apoptosis in response to DNA damage. The complex p53–p63 inhibits the activity of Rab7 – a protein involved in the degradation of EGFR which induces cell proliferation, angiogenesis, and inhibits apoptosis.⁷⁴ Available evidence suggests a possible relationship between p53 and *BRCA1*, with an increased frequency of p53 mutations in *BRCA*-related tumors.³ p53-mutated tumors are characterized by aggressive tumor biology with poor differentiation, high tumor grade, and invasiveness.^{75,76} p53 overexpression seems to be associated with a worse prognosis in terms of OS and DFS, but with higher chemotherapy sensitivity.^{75,77–80} With regard to TNBC, many studies have demonstrated that p53 is mutated in the majority (60%–88%) of these tumors.⁸¹ The most frequent p53 mutations in TNBC are missense mutations with single-amino-acid substitution.⁸² Bidard et al showed that a p53+/TNBC tumor treated with anthracyclines/alkylating agents had a higher probability of achieving pCR compared to other p53+/BC subtypes ($p < 0.001$).⁸³

Chemotherapy agents in the neoadjuvant setting

Cytotoxic chemotherapy is the backbone of TNBC treatment. In a large study evaluating the neoadjuvant setting, Liedtke et al evaluated the response to different neoadjuvant chemotherapy regimens in 1,118 patients with early-stage BC treated with different drugs.⁸⁴ In the subgroup of TNBC, the pCR rate was higher as compared to that among non-TNBC patients (22% vs 11%; $p = 0.034$), independent of chemotherapy regimens.⁸⁴ Actually, the standard of care in the neoadjuvant setting for TNBC is sequential anthracycline–taxane-based chemotherapy.⁵ The pCR rate of these regimens ranges from 28% to 36%.^{84–86} To increase the rate of response, different chemotherapeutic strategies have been tested (Table 2).

Weekly nanoparticle-albumin-bound paclitaxel (nab-paclitaxel) instead of weekly paclitaxel

Available evidence suggests a possible clinical benefit in terms of tumor response due to the introduction of nab-paclitaxel instead of weekly paclitaxel. In the phase III GeparSepto study, pCR was reached in 48% of TNBC patients treated with weekly nab-paclitaxel 150 mg/m² versus 26% of patients treated with weekly paclitaxel 80 mg/m² ($p = 0.00027$).⁸⁷ Of note, the dose of nab-paclitaxel was reduced from 150 to 125 mg/m² due to the higher incidence of severe sensory neuropathy, without affecting the treatment efficacy.⁸⁷ A phase III trial (ETNA study) first presented at the the American Society of Clinical Oncology (ASCO) 2016 showed a higher rate of response in the nab-paclitaxel arm compared to the paclitaxel one (41.3% vs 35.5%; p -value not statistically significant).⁸⁸ Similar pCR rates have been reported by Kuwayama et al in a subgroup of TNBC patients treated with four cycles of weekly nab-paclitaxel 100 mg/m².⁸⁹ Moreover, early results from the WSG-ADAPT trial showed a possible advantage in terms of pCR by adding nab-paclitaxel, rather than gemcitabine, to carboplatin (44.5 vs 28.4, $p = 0.004$).²² Several clinical trials are currently further investigating the role of nab-paclitaxel in this setting.

Addition of platinum agents

The hypothesis of an increased efficacy of platinum agents in TNBC is based on the fact that these tumors often show functional *BRCA1* alterations that increase the sensitivity to cross-linking agents.⁹⁰ Clinical evidence suggests that the addition of platinum to standard chemotherapy increases

Table 2 pCR rate due to different chemotherapy regimens reported in published clinical trials in TNBC patients

| Study | Study design | TNBC n | Treatment | pCR definition | pCR % |
|------------------------------------|-----------------|--------|--|----------------|-------|
| Anthracycline/taxane | | | | | |
| Liedtke et al ⁸⁴ | Prospective | 255 | Taxane + anthracycline + cyclophosphamide + fluorouracil | ypT0/is ypN0 | 28 |
| von Minckwitz et al ⁸⁵ | Pooled analysis | 742 | Anthracycline + taxane | ypT0/is ypN0 | 34 |
| von Minckwitz et al ⁸⁶ | Pooled analysis | 911 | Anthracycline + taxane | ypT0/is ypN0 | 35.8 |
| Carboplatin | | | | | |
| Sikov et al ⁹⁵ | Phase II | 12 | Paclitaxel + carboplatin | ypT0/is ypN0 | 67 |
| Chen et al ⁹⁶ | Phase II | 17 | Paclitaxel + carboplatin | ypT0/is ypN0 | 33.3 |
| Roy et al ⁹⁷ | Phase II | 9 | Docetaxel + carboplatin | ypT0 ypN0 | 44 |
| Chang et al ⁹⁸ | Phase II | 11 | Docetaxel + carboplatin | ypT0/is ypN0 | 55 |
| Campos Gomez et al ⁹⁹ | Phase II | 35 | Doxorubicin + cyclophosphamide → docetaxel + carboplatin | ypT0/is ypN0 | 50 |
| Von Minckwitz et al ¹⁰⁰ | Phase III | 158 | Paclitaxel + liposomal doxorubicin + bevacizumab + carboplatin | ypT0 ypN0 | 53.2 |
| Nab-paclitaxel | | | | | |
| Untch et al ⁸⁷ | Phase III | 139 | Nab-paclitaxel → epirubicin + cyclophosphamide | ypT0 ypN0 | 48 |
| Gianni et al ⁸⁸ | Phase III | 219 | Nab-paclitaxel + anthracycline (investigator choice) | ypT0/is ypN0 | 41.3 |
| Gluz et al ²² | Phase II | 61 | Nab-paclitaxel + carboplatin | ypT0/is ypN0 | 49.2 |
| Kuwayama et al ⁸⁹ | Phase II | 54 | Nab-paclitaxel + anthracycline + cyclophosphamide + fluorouracil | ypT0/is ypN0 | 30 |
| Eribulin | | | | | |
| Kaklamani et al ¹⁰² | Phase II | 30 | Carboplatin + eribulin | ypT0/is ypN0 | 43 |

Abbreviations: TNBC, triple negative breast cancer; pCR, pathological complete response; Nab-paclitaxel, nanoparticle-albumin-bound paclitaxel.

the rate of tumor response, but this clinical advantage has not been clearly translated into a survival benefit. Platinum-based chemotherapy has been investigated in several studies, but the best platinum agent, the ideal combination, or the best sequence with other chemotherapy agents remains unknown.^{91–93} In particular, a retrospective study that compared docetaxel with cisplatin or carboplatin showed the superiority of cisplatin in terms of OS (hazard ratio [HR] 0.49, $p = 0.007$) and PFS (HR 0.40, $p = 0.018$) with a quite good tolerability profile.⁹² This difference between the two platinum agents was not confirmed in another large pooled analysis.⁹⁴ More consistent are the data on the increased response rate due to the addition of platinum to standard chemotherapy, mainly in anthracycline-free regimens.^{95–98} The subgroup analysis of five clinical trials, conducted in TNBC patients treated with neoadjuvant platinum-containing therapies combined with taxanes, showed a pCR rate ranging from 33% to 67%.^{95–99} These data are in accordance with the results from the GeparSixto study, where the addition of carboplatin to anthracycline or taxane-based therapy improved the pCR from 36.9% to 53.2%.¹⁰⁰ A large meta-analysis (included six randomized controlled trials and 22 retrospective or prospective studies) strongly confirmed the advantage due to platinum addition in both the objective response rate (86.7%) and the pCR rate (48.4%).⁹⁴ Data presented at the ASCO 2017 further underline the advantage in tumor response in patients treated with carboplatin/docetaxel compared to taxane–anthracycline-based regimen.¹⁰¹ Data from the WSG-

ADAPT TN trial suggested that immune marker gene expression (CD8, PD1, and PFDL1) and high-proliferation markers (proliferation score, Prosigna Breast Cancer Prognostic Gene Signature Assay [PAM-50] risk-of-recurrence [ROR] score, MKI67, CDC20, NUF2, KIF2C, CENPF, EMP3, and TYMS) were positively associated with pCR in carbo-containing chemotherapy regimens.²² In all of these reported studies, the addition of carboplatin increased hematological and non-hematological toxicity, with consequent frequent dose reduction. Nowadays, the increased toxicity and the lack of clear demonstrated long-term survival benefit due to platinum addition constitute reasons why platinum agents are not included in the standard of care for TNBC in the neoadjuvant setting. Despite this, the evidence that the pCR is strongly associated with OS in TNBC is an argument often used by the physicians to justify the addition of carboplatin to NST in clinical practice. Several phase III trials are ongoing and may provide more information on this topic.

Eribulin in addition to carboplatin

Eribulin is a non-taxane microtubule inhibitor that causes irreversible cell-cycle blockade at the G2–M phase. Eribulin is approved in the management of metastatic BC after at least two treatment regimens with an anthracycline and a taxane, but its role in other settings is currently under investigation. A phase II clinical trial conducted in 30 TN early-stage BC patients investigated the response rate to 3-weekly carboplatin AUC 6 plus eribulin 1.4 mg/m² (days 1 and 8 every 21 days).¹⁰² In

total, 80% of enrolled patients had a clinical complete or partial response, and 43% achieved pCR.¹⁰² Moreover, another phase II international trial (NCT01372579) is ongoing with the aim to investigate the efficacy of a preoperative eribulin mesylate and carboplatin combination in stages I–III TNBCs.¹⁰³

Dose-dense chemotherapy

Dose-dense (DD) chemotherapy aims to achieve maximum tumor death by delivering therapeutic drugs over a shorter duration. The efficacy of DD systemic treatment was mainly investigated in the adjuvant setting. A recent meta-analysis including a total of eight phase III trials (17,188 randomized women) showed that patients treated with DD chemotherapy had better OS and DFS than those on the conventional schedule. In particular, statistically significant OS benefit was observed in patients with hormone-receptor-negative tumors (HR 0.8, $p = 0.002$).¹⁰⁴ Data from the DD schedule in the neoadjuvant setting are less consistent.¹⁰⁵ Published clinical trials included women treated with outdated regimens and/or schedules; there are no data regarding the benefit of DD anthracycline–taxane-based primary chemotherapy. Conventionally, in clinical practice, results from DD adjuvant chemotherapy studies are applied in the neoadjuvant setting too.

Targeted agents tested in neoadjuvant setting

Table 3 summarizes the main clinical trials where targeted agents have been evaluated in addition to NST.

PARP-I inhibitors

PARP-1 is an enzyme known to be involved in the base-excision repair pathway, which plays a key role in the repair

of single-stranded DNA breaks.^{106,107} During the last decade, drugs able to interfere with the DNA-damage-repair systems and to induce a synthetic lethality, named PARP inhibitors (such as iniparib, olaparib, and veliparib), have been developed. The main evidence is in the metastatic setting, where PARP inhibitors have been tested as single agents and in combination with chemotherapy.^{9,108} The best results in terms of efficacy emerged from combination with cisplatin or carboplatin, as well as with topotecan and temozolamide, with response rates in BRCA-related BC of up to 73%.^{109–112} Considering the use of PARP inhibitors in the neoadjuvant setting, a single-arm phase II study showed efficacy in terms of pCR of gemcitabine combined with carboplatin and iniparib in TNBC.¹¹³ In this study, the presence of HRD was associated with higher response rates, regardless of *BRCA 1/2* mutational status.¹¹³ Results from the veliparib and carboplatin arm in the I-SPY-2 trial, a multicenter, adaptively randomized trial, reported 51% pCR in the experimental arm versus 26% in the standard regimen. Considering adverse events, hematological side effects were higher in the veliparib–carboplatin group than in the control arm.¹¹⁴ No difference in the pCR rate due to the addition of veliparib to carboplatin–paclitaxel, followed by doxorubicin plus cyclophosphamide, has been reported in a phase III study presented by the German Breast Group at the ASCO 2017.¹¹⁵ A phase II study of neoadjuvant talazoparib monotherapy in BRCA-associated BC is ongoing at the MD Anderson Cancer Center.¹¹⁶ Considering these preliminary controversial results, PARP inhibitors are still under investigation.

Anti-angiogenic agents

Bevacizumab is a humanized monoclonal antibody that targets the main isoforms of circulating vascular endothelial

Table 3 pCR rate TNBC patients treated with chemotherapy plus targeted agents

| Study | Study design | TNBC n | Treatment | pCR definition | pCR % |
|-------------------------------|--------------|--------|---|----------------|-------|
| Bevacizumab | | | | | |
| Gerber et al ¹⁷ | Phase III | 323 | Epirubicin + cyclophosphamide → docetaxel + bevacizumab | ypT0 ypN0 | 39 |
| Earl et al ¹²¹ | Phase III | 119 | Bevacizumab + docetaxel → epirubicin + cyclophosphamide | ypT0/is ypN0 | 45 |
| Sikov et al ¹²² | Phase II | 226 | Paclitaxel → doxorubicin + cyclophosphamide + bevacizumab | ypT0/is* | 59 |
| Guarneri et al ¹²⁴ | Phase II | 44 | Paclitaxel + carboplatin + bevacizumab | ypT0/is ypN0 | 50 |
| Kim et al ¹²⁵ | Phase II | 45 | Carboplatin + docetaxel + bevacizumab | ypT0/is ypN0 | 42 |
| Nahleh et al ¹²⁶ | Phase II | 32 | Nab-paclitaxel + bevacizumab → adriamycin + cyclophosphamide | ypT0/is ypN0 | 59 |
| Mrózek et al ¹²⁷ | Phase II | 12 | Nab-paclitaxel + carboplatin + bevacizumab | ypT0 ypN0 | 50 |
| PARP-inhibitor | | | | | |
| Telli et al ¹¹³ | Phase II | 80 | Gemcitabine + carboplatin + iniparib [§] | ypT0/is ypN0 | 36 |
| Rugo et al ¹¹⁴ | Phase II | 54 | Veliparib + carboplatin → doxorubicin + cyclophosphamide | ypT0 ypN0 | 51 |
| Immunotherapy | | | | | |
| Schmid et al ¹²⁸ | Phase IB | 20 | Pembrolizumab + nab-paclitaxel → pembrolizumab + doxorubicin + cyclophosphamide ± carboplatin | ypT0/is ypN0 | 85 |
| Nanda et al ¹²⁹ | Phase II | 21 | Paclitaxel + pembrolizumab → doxorubicin + cyclophosphamide | ypT0/is ypN0 | 71 |

Note: *pCR in breast only; [§]pCR rate in patients treated with six cycles of neoadjuvant treatment.

Abbreviations: TNBC, triple negative breast cancer; pCR, pathological complete response.

growth factor (VEGF), resulting in the inhibition of angiogenesis, cell tumor growth, and cell survival.¹¹⁷ Bevacizumab use has been investigated in both advanced and early-stage BC treatments, showing an increased response rate – mainly, in TNBC patients.^{84,100,118–120} When added to chemotherapy in preoperative treatment, the pCR rate ranges from 40% to 59% independent of the chemotherapy regimen administered. The phase III GeparQuinto trial included 663 TNBC patients treated with epirubicin and cyclophosphamide followed by docetaxel with or without bevacizumab, and showed a pCR rate of 39.3% in the bevacizumab arm versus 27.9% in the control arm ($p = 0.021$).⁷ The advantage in response rate due to the addition of bevacizumab has been demonstrated in two other large trials. The first one is the multicenter British phase III study (ARTemis), where 781 patients were randomized to receive bevacizumab or placebo plus docetaxel followed by cyclophosphamide, 5-fluorouracil, and epirubicin.¹²¹ In the TNBC subgroup, bevacizumab provided an advantage in terms of pCR from 34% to 49%.¹²¹ The second one – the CALGB 40603/Alliance trial, conducted in 443 patients with stages II and III TNBC – confirmed the increase of tumor response in the bevacizumab group, independent of the chemotherapy regimen administered.^{122,123} Similar response rates have been found in two phase II trials: the Ca.Pa.Be study, where 44 TNBC women were treated with a combination of paclitaxel, carboplatin, and bevacizumab, and the KCSG BR-0905 trial, where 45 women were treated with bevacizumab, docetaxel, and carboplatin.^{124,125} The addition of bevacizumab to nab-paclitaxel was investigated in two phase II trials, showing an increase of response rate compared to bevacizumab plus paclitaxel or docetaxel.^{126,127} In the first one, the SWOG S0800 trial, the combination of bevacizumab plus nab-paclitaxel, followed by DD doxorubicin and cyclophosphamide, increased the pCR rate up to 59% ($p = 0.014$).¹²⁶ In the second one, 50% of TNBC patients treated with bevacizumab plus nab-paclitaxel and carboplatin achieved pCR.¹²⁷ With regard to safety profile, patients treated with bevacizumab experienced an increased number of immediate and delayed postoperative complications as well as neutropenia and hypertension.^{121,122} Nowadays, the use of bevacizumab in the neoadjuvant setting is still controversial and not recommended, mainly due to the lack of survival-benefit evidence.

Immunotherapy

The evidence that stimulating the immune cells might, therefore, be an option to increase response rates is the rationale for designing clinical trials with the addition of immunotherapy

in the neoadjuvant setting. Preliminary results from the KEYNOTE-173 presented at the ASCO 2017 (nab-paclitaxel ± carboplatin plus pembrolizumab, followed by cyclophosphamide and doxorubicin) suggested promising antitumor activity of pembrolizumab when combined with NST.¹²⁸

The objective response rate before surgery was 100% in the pembrolizumab and carboplatin group versus 80% in the other experimental group (nab-paclitaxel + pembrolizumab); the pCR rate (yT0/Tis yN0) was 90% versus 60%, respectively.¹²⁸ Similar results have been reported in the subgroup of patients treated with pembrolizumab in the I-SPY-2 trial.¹²⁹ Data presented at the ASCO 2017 were based on results observed in patients at high risk of relapse using upfront tumor profiling (including mammaPrint 70-gene signature test). Patients were treated with weekly paclitaxel for 12 weeks ± pembrolizumab, followed by anthracyclines.¹²⁹ In the TNBC women, an absolute increase in the estimated pCR rate of 40% was observed in the pembrolizumab arm (based on the estimated pCR rate of 60% with pembrolizumab plus standard therapy compared to 20% with standard NST alone).¹²⁹ Currently, two different strategies are under investigation to evaluate the real benefit of immunotherapy in early-stage TNBC: the addition of PD1/PD-L1 inhibitors to different NST regimens and the administration of PD1/PD-L1 inhibitors in the adjuvant setting in patients with residual BC disease after NST. In the first case, four studies are currently recruiting patients: the phase III NeoTRIPaPDL1 trial, a multicenter randomized study with patients treated with nab-paclitaxel plus carboplatin ± atezolizumab;¹³⁰ the GeparNuevo trial, a phase II study of nab-paclitaxel ± durvalumab followed by epirubicin plus cyclophosphamide; and two other studies with nab-paclitaxel plus atezolizumab or durvalumab.^{131–133} With regard to the second strategy, two big clinical trials are now ongoing, both conducted in high-risk TNBC patients with residual disease after NST, randomized to receive 1 year of adjuvant pembrolizumab or avelumab versus observation.^{134,135} The results of all these studies are awaited with high interest.

Conclusion

According to current international guidelines, in early-stage TNBC, the timing of treatment (pre- vs postoperative) has no effect on long-term outcomes. All chemotherapy strategies used in adjuvant treatment may also be used preoperatively. If a primary chemotherapy treatment is used, it is recommended to deliver all planned treatment without unnecessary breaks, irrespective of the magnitude of tumor response.^{136,137}

In our opinion, NST should be the first option in the case of operable TNBC, especially in locally advanced cases

(when mastectomy is required due to tumor size), as well as in high-grade and high-proliferation-rate tumors. Despite the progress in our understanding of TNBC, anthracycline–taxane-based chemotherapy remains the standard of care for NST in all TNBC subtypes. Platinum agents, as well as PARP-inhibitor agents, provide pCR advantage in different clinical trials, mainly in BRCA-defense tumors, without demonstrating an improvement in survival benefit. At present, there are no clear predictive biomarkers useful in clinical practice. The classification of TNBC using molecular profile showed how TNBCs are a heterogeneous group which explains the lack of survival benefit of experimental drugs tested in several clinical trials. All of the available evidence suggests the necessity of design biology-driven clinical trials wherein TNBC patients may be treated on the basis of tumor molecular profile.

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The authors report no conflicts of interest in this work.

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