Management of triple negative breast cancer

Catherine Oakman, Giuseppe Viale, Angelo Di Leo

Triple negative breast cancer (TNBC) accounts for approximately 15% of breast cancer cases. TNBC is an immunohistochemically defined subtype, with significant diversity within the subtype. Generally TNBC occurs in younger women and is marked by high rates of relapse, visceral and CNS metastases, and early death. Current therapy fails to curtail the innate aggressive behaviour of TNBC in the majority of patients.

TNBC is not one disease, but a common IHC status for a number of tumours with heterogenous biology and clinical behaviour. See Artifl. 1 for details.

It is critical to recognise that TNBC is a heterogeneous disease, for which chemotherapy alone is inadequate for the majority of patients. New treatment options are urgently required.

Reliability and reproducibility are key considerations. Variable immunohistochemistry (IHC) factors include tissue preparation, choice of antibody, detection of antibody and interpretation of results, which in addition may be compounded by lack of inter-laboratory reproducibility. IHC staining is associated with a remarkable rate of discordance for both ER, PR and HER2.

TNBC is an immunohistochemical description of breast cancers with a triplet of negative staining for ER, PR and HER2. In contrast, the basal-like breast cancer phenotype is molecularly defined. Critical to this immunohistochemical definition of TNBC is clarity regarding 'negative' staining. Prior arbitrary thresholds for discriminating between positive and negative ER and PR status include 1%, 10% and 20%. Diagnostic thresholds vary between laboratories and clinical trials, making cross trial comparison and application of results difficult. Clarity is offered by recently published San Gallen guidelines which define endocrine responsiveness as any staining, indeed ≥1%.

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Subtypes within the subtype

TNBC is not one disease, but a common IHC status for a number of tumours with heterogenous biology and clinical behaviour. See Artifl. 1 for details.
phenotype. The term basal-like refers to breast cancers with molecular breast cancer subtypes, one of which is the basal-like phenotype. A pivotal report by Perou et al. identified five distinct molecular breast cancer subtypes, one of which is the basal-like phenotype. The term basal-like refers to breast cancers with a gene expression profile resembling normal breast basal/myoepithelial cells, characterised by expression of basal cytokeratins (CK5/6, CK14, CK17), caveolin 1 and 2, cyclin-D1, vimentin and p-cadherin, and lack of expression of ER, PR and HER2. It is tempting to assume that TNBC and basal-like breast cancer are synonymous, but they are not. Only 71–91% of TNBC have a basal-like phenotype. Only 77% of basal-like cancers are TNBC, i.e. more than 20% of basal-like cancers are non-TNBC. In the absence of clinical sample gene phenotyping for the basal-like subgroup, further IHC markers have been suggested as surrogates for identifying the basal-like phenotype using IHC criteria. CK5, CK6 and EGFR are commonly cited as potential surrogates. Other contenders are numerous and include ki-67, CK14, CK17, CK21. However variable expression, lack of standardised thresholds and lack of validation limit their use. A ‘five-marker’ approach proposed by Nielsen et al., defined immunohistochemically as triple negative and cytokeratin 5/6 and/or EGFR positive, is often used as a surrogate for basal-like disease.

Beyond the basal phenotype, there is also substantial overlap between TNBC and BRCA1 mutated tumours. Ninety percent of TNBC have a basal-like phenotype. Only 77% of basal-like cancers are TNBC, i.e. more than 20% of basal-like cancers are non-TNBC. In the absence of clinical sample gene phenotyping for the basal-like subgroup, further IHC markers have been suggested as surrogates for identifying the basal-like phenotype using IHC criteria. See Fig. 1. A pivotal report by Perou et al. identified five distinct molecular breast cancer subtypes, one of which is the basal-like phenotype. The term basal-like refers to breast cancers with a gene expression profile resembling normal breast basal/myoepithelial cells, characterised by expression of basal cytokeratins (CK5/6, CK14, CK17), caveolin 1 and 2, cyclin-D1, vimentin and p-cadherin, and lack of expression of ER, PR and HER2. It is tempting to assume that TNBC and basal-like breast cancer are synonymous, but they are not. Only 71–91% of TNBC have a basal-like phenotype. Only 77% of basal-like cancers are TNBC, i.e. more than 20% of basal-like cancers are non-TNBC. In the absence of clinical sample gene phenotyping for the basal-like subgroup, further IHC markers have been suggested as surrogates for identifying the basal-like phenotype using IHC criteria. CK5, CK6 and EGFR are commonly cited as potential surrogates. Other contenders are numerous and include ki-67, CK14, CK17, CK21. However variable expression, lack of standardised thresholds and lack of validation limit their use. A ‘five-marker’ approach proposed by Nielsen et al., defined immunohistochemically as triple negative and cytokeratin 5/6 and/or EGFR positive, is often used as a surrogate for basal-like disease.

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Clinical approach

Perhaps the best approach for defining TNBC, which is also feasible and cost effective, is not to switch or substitute detection methods, but to use available methods in a complimentary hierarchical approach. Morphology alone may define some tumour subtypes that will be triple negative. Next, IHC with standardised guidelines will provide ER/PR/HER2 status. Finally, for cases in whom further information would impact on management, future use of validated surrogate IHC markers or gene profiling assays may refine diagnosis.

Treatment

Due to underlying biological heterogeneity within TNBC, the concept of a standard approach is inappropriate. Some patients have excellent outcome in the absence of adjuvant therapy, some are cured by adjuvant cytotoxic use and some have a grim outlook regardless of administration of currently available systemic treatment.

A subgroup of TNBC have long term disease-free survival (DFS) in the absence of adjuvant systemic therapy. A study comparing 5-year local regional relapse, distant metastases free survival (DMFS) and cause specific survival (CSS) between IHC defined breast cancer subtypes revealed no significant difference in local control, however TNBC was associated with poorer DMFS and CSS compared with non-TNBC. Of note in this study, whilst TNBC patients as a group had poor outcome, some TNBC patients remained disease-free after 5 years. Of 40 TNBC patients treated with surgery and radiotherapy alone, DMFS and CSS were 82% and 86% respectively. Whilst most of these patients had small (T1), node negative disease, their outcome was good despite triple negative status.

Clinical studies, particularly in the neoadjuvant setting, have highlighted that a subset of patients with early TNBC have highly chemosensitive disease with excellent long term outcome.
patients have high rates of pathological complete response (pCR) to neoadjuvant chemotherapy, higher than non-TNBC. See Table 2. Prospective gene expression profiling in the neoadjuvant setting reported diverse chemosensitivity between and within breast cancer subtypes.\textsuperscript{21} Molecularly defined basal-like disease, as well as the HER2-positive subgroup, were associated with increased pCR. Within basal-like disease, a differentially expressed multigene set correlated with pCR and has potential in predicting pCR within the subgroup.

pCR correlates with excellent long term DFS and overall survival (OS).\textsuperscript{2,20,33} However, the majority of early TNBC patients have a poor outcome despite systemic therapy due to chemoresistant micrometastatic disease and subsequent early, aggressive relapse, within 3 years from primary diagnosis and with a short interval from distant recurrence to death (median 13 months).\textsuperscript{3,20,36} Thus, the reported paradox for TNBC patients from neoadjuvant trials: higher pCR rate yet worse OS compared with non-TNBC.\textsuperscript{20}

This discordance between pCR and OS is highlighted by 2 studies. A single institution prospectively created a database of neoadjuvant chemotherapy in 1118 early breast cancer patients, including 255 TNBC.\textsuperscript{3} Various chemotherapy schedules, generally anthracycline and taxane based, induced pCR in 22% and 11% of TNBC and non-TNBC patients, respectively (Odds ratio (OR) = 1.53; 95% Confidence interval (CI): 1.03–2.26; p = 0.34), but significantly decreased 3-year progression free survival (PFS) (63% vs. 76%, respectively; Hazard ratio (HR) 1.86; 95% CI 1.39–2.50, p < 0.0001). Importantly, all patients with pCR had excellent survival, independent of IHC subtype. However TNBC patients with residual disease had reduced OS compared with non-TNBC, particularly in the first 3 years (p < 0.0001). Similarly, examination of pCR rates from neoadjuvant anthracycline, 2 or 3 weekly schedule, with or without taxane therapy in different molecular classes of breast cancer revealed this discordance.\textsuperscript{20} From 107 patients, 34 had TNBC (32%). A high pCR was reported in the TNBC patients (27% vs. 11% for non-TNBC; p = 0.01) but significantly decreased distant disease-free survival and OS. The worse outcome was due to early relapse in patients with residual disease.

TNBC patients do not have the option of endocrine or anti-HER2 therapy as they lack the targets for these agents. In the absence of targeted treatment, the current option is chemotherapy. Patients with chemosensitive disease lack a standardised approach. Patients with cytotoxic resistant disease are in urgent need of new therapies.

**Chemotherapy**

Despite consensus regarding increased chemosensitivity in TNBC, there is no agreement regarding optimal choice or schedule of cytotoxics. Most evidence derives from retrospective, subgroup analyses with small patient numbers and inadequate power. Whilst most clinicians would currently employ an intensive approach, including an anthracycline and a taxane, and the notion of DNA damaging platinums is gaining popularity, there is currently no consensus regarding the optimal choice or schedule of cytotoxics in TNBC patients.

Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Detection method</th>
<th>Regimen</th>
<th>No. of TNBC pts</th>
<th>TNBC pCR (%)</th>
<th>Non-TNBC pCR (%)</th>
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<tr>
<td>2007</td>
<td>IHC</td>
<td>Overall</td>
<td>96</td>
<td>29</td>
<td>13</td>
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<td></td>
<td>- Intensified FAC</td>
<td>-56</td>
<td>-47</td>
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<td></td>
<td></td>
<td>- FEC</td>
<td>-40</td>
<td>-13</td>
<td></td>
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<tr>
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<td>IHC</td>
<td>FAC or FEC</td>
<td>120</td>
<td>17</td>
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<td>Molecular</td>
<td>T-FAC</td>
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<td>45</td>
<td>18</td>
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<td>IHC</td>
<td>Anthracycline + taxane</td>
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<td>39</td>
<td>12</td>
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<tr>
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<td>IHC</td>
<td>AC +/- taxane</td>
<td>34</td>
<td>27</td>
<td>11</td>
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<td>Docetaxel + Doxorubicin</td>
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<td>17</td>
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<tr>
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<td>IHC</td>
<td>Overall</td>
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<td>22</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>- FAC/FEC/AC</td>
<td>-70</td>
<td>-20</td>
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<td></td>
<td>- T-FAC/T-FEC</td>
<td>-125</td>
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<td></td>
<td></td>
<td>- Single agent taxane</td>
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<td>- Other</td>
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<td>Molecular</td>
<td>AC → Paclitaxel</td>
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<td>34</td>
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<td>Anthracycline + taxane</td>
<td>21</td>
<td>38</td>
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<td>38</td>
<td>34</td>
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<td>22</td>
<td>23</td>
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<tr>
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<td>Carboplatin + paclitaxel</td>
<td>12</td>
<td>67</td>
<td>39</td>
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<td>IHC</td>
<td>E/Cis/F → Paclitaxel</td>
<td>30</td>
<td>40</td>
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<tr>
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<td>IHC</td>
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<td>90</td>
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<td>IHC</td>
<td>Ixabepolone</td>
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<td>19</td>
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\textsuperscript{a} Prospective study.

\textsuperscript{b} Clinical complete response, not pathological complete response.

\textsuperscript{c} Of 10 patients, all had BRCA1 mutation and 9 of 9 with known IHC status had TNBC. AC: doxorubicin/cyclophosphamide; AT: doxorubicin/docetaxel; E/Cis/F: epirubicin/cisplatin/5-fluorouracil; FAC: 5-fluorouracil/doxorubicin/cyclophosphamide; FEC: 5-fluorouracil/epirubicin/cyclophosphamide; IHC: immunohistochemistry; n/ap not applicable; n/av not available; pCR: pathological complete response; T: paclitaxel; TNBC: triple negative breast cancer.
limited prospective evidence to support these approaches in TNBC populations.

**Anthracyclines**

The benefit of anthracycline-based therapy in TNBC is undefined. Theoretically anthracyclines would be ideal in tumours with overexpression of the anthracycline drug target, topoisomerase IIα, and aberrant DNA repair. Whilst topoisomerase IIα gene amplification is exceptionally rare in TNBC, if it occurs at all, overexpression of the topoisomerase IIα protein is common due to high proliferative signalling which upregulates protein expression. 17 Unlike HER2 for example which shows strong correlation between level of gene amplification and protein overexpression, 12 topoisomerase IIα shows variable correlation between gene status and protein levels due to complex, multifactorial regulation of transcription, translation and mRNA stabilization. 37 A critical regulator of transcription is the cellular proliferative rate, evidenced by a strong correlation between proliferation markers and topoisomerase IIα protein levels, and cell-cycle phase dependence of topo IIα mRNA transcription. 38

TNBC is marked by a high rate of BRCA mutation or epigenetic silencing with impaired DNA repair. 19 Loss of BRCA, or BRCA dysfunction in the absence of gene mutation, 39 may impart particular sensitivity to DNA damaging agents. Thus, overexpression of topoisomerase IIα and dysfunctional DNA repair provide biological rationale in support of anthracycline benefit in TNBC. Most available clinical data regarding anthracycline benefit comes from retrospective, underpowered subgroup analyses and meta-analyses.

Retrospective analysis was performed on 96 TNBC patients from 2 prospective anthracycline-based, neoadjuvant trials. 21 Patients received either standard fluorouracil (500 mg/m²), epirubicin (100 mg/m²) and cyclophosphamide (500 mg/m²) (FEC100) or intensified fluorouracil (700 mg/m²), doxorubicin (70 mg/m²) and cyclophosphamide (700 mg/m²) (FAC). Overall pCR rate was 29%. Within the 2 schedules however, marked difference in pCR was observed for TNBC with pCR rates of 47% and only 13%, for FAC and FEC respectively, which may be attributable to either intensification of anthracycline and/or intensified cyclophosphamide. Another retrospective analysis of 293 patients, 120 of whom had TNBC, assessed pCR following neoadjuvant FAC. 22 The TNBC cohort had pCR of 17% compared with 4% in non-TNBC (p = 0.0004).

Two meta-analyses report restriction of anthracycline benefit to patients with HER2 overexpression. 40, 41 These results however are limited by heterogeneity within the HER2-positive and HER2-negative subtypes, use of published summary results rather than individual patient data and diversity in anthracycline drug, dose and scheduling between trials. Another recent meta-analysis suggests that in addition to anthracycline benefit seen in HER2-positive disease, benefit may extend to the HER2-negative population, specifically TNBC. 42 A planned exploratory DFS analysis by molecular subgroups (ER, PgR, grade, HER2) identified 294 TNBC patients, for whom anthracycline-based therapy appeared superior to cyclophosphamide, methotrexate and 5-fluorouracil (CMF) (DFS HR: 0.77; 95% CI 0.54–1.09).

Conversely, TNBC patients with a basal phenotype have been reported to have inferior outcome from anthracycline- vs. non anthracycline-based therapy. 43 Recent reanalysis of the Canadian NCIC-CTG MA5 trial comparing CMF and cyclophosphamide, epirubicin and 5-fluorouracil (CEF) in premenopausal, node positive women used IHC to subdivide 511 of the total 710 trial patients into biological subgroups. The two treatment variables in the CEF arm compared with CMF were inclusion of epirubicin and reduction in cyclophosphamide dose. Core basal phenotype was defined by the five-marker method' (ER-, PR-, HER2- and CK5/6- or EGFR+). CEF was less efficacious then CMF in the triple negative core basal group (5-year OS 51% vs. 71%, respectively). In the triple negative non basal group, 5-year OS were similar between groups (65% and 63%) but small patients numbers (n = 29) prevent meaningful conclusions. Thus, reduction of cyclophosphamide and inclusion of an anthracycline appears inferior to classical CMF in TNBC.

Thus, available results conflict. No current prospective trial will answer the question of anthracycline efficacy in TNBC. Omission of anthracycline in patients with HER2-negative disease, namely docetaxel and cyclophosphamide (TC) vs. docetaxel, adriamycin and cyclophosphamide (TAC), is under trial by the US Oncology Trials Group. 44 However, as HER2-negative disease is dominated by ER positive, HER2-negative luminal breast cancer, any hypothesized benefit in the TNBC minority will likely be confounded by relative chemo resistance in the majority of patients with luminal disease and a favourable prognosis. Trials to define the role of anthracyclines specifically in TNBC patients are needed.

**Taxanes**

Clinical evidence for taxane use specifically in TNBC is limited and inconclusive. A potential rationale for taxane benefit in TNBC is a link between p53 mutations and taxane benefit. Mutations of the critical tumour suppressor gene, p53, occurs in approximately 80% of TNBC. Whilst evidence for mutant p53 as a predictive marker for taxanes is conflicting, intriguing preclinical and clinical work supports taxane benefit despite p53 mutation, suggesting a p53-independent mode of action for taxanes. 45–47

The BCIRG 001 trial compared adjuvant TAC with FAC in node positive patients. An unplanned retrospective subset analysis of 1350 patients, 91% of total patients in the trial, investigated the prognostic and predictive significance of centrally confirmed IHC subtyping, based on ER, PR, HER2 and ki-67. 36 Subgroups were found to predict DFS and OS. For 192 patients (14.5%) with TNBC, 3-year DFS was 67%, compared with 68%, 82% and 91% for the HER2, luminal B and luminal A subtypes, respectively. In the TNBC subtype, a trend for benefit from TAC over FAC was reported, with 3-year DFS of 73.5% and 60% respectively (p = 0.051). The TNBC subtype had a worse OS, marked by rapid relapse within the first 3 years post primary diagnosis. The CALGB 9344 trial assessed the addition of paclitaxel to cyclophosphamide and adriamycin in node positive early breast cancer patients, with incremental taxane benefit for DFS and OS for the trial population. Retrospective evaluation of these patients reported minimal incremental taxane benefit in the predominant luminal subgroup, with the greatest benefit in the HER2-positive and ER/HER2-negative subtypes. 48 PACS 01 compared 6 cycles of FEC with 3 cycles of FEC followed by 3 cycles of docetaxel in nearly 2000 women with node positive operable breast cancer. Retrospective analysis of 1100 patients using a unique 33 marker IHC panel to define breast cancer subtypes revealed additional benefit from the taxane over FEC therapy alone in patients with a basal-like profile as defined by the IHC panel. 49 Conversely the luminal subtypes exhibited no differential efficacy between treatment arms. The TACT trial compared 4 cycles of FEC followed by 4 cycles of docetaxel with an anthracycline-based, non taxane control arm in over 4000 women with node positive or high risk node negative early breast cancer. In the trial population, no overall benefit was shown from addition of docetaxel to standard therapy. In contrast to CALGB 9433 and PACS 01, the TACT exploratory subgroup analysis did not show any additional benefit from the taxane in the ER/HER2-negative subgroup, even when the subgroup was further refined to ER/HER2-negative with lymph node positive disease. 50
Prior to commencing neoadjuvant paclitaxel followed by FAC (T/FAC), fine needle aspirates were taken from 82 patients with breast cancer for gene expression profiling. Both the basal-like and HER2-positive subtypes had a pCR rate of 45%. In contrast, luminal tumours had a pCR rate of 6%. As has been highlighted, the basal phenotype is not synonymous with TNBC, but the substantial overlap makes this result promising for further investigation of taxane based therapy in the triple negative subset.

Platinum

Platinum-based compounds have not been found to benefit the majority of breast cancer patients. However few trials to date have specifically explored platinums in TNBC. Indeed background data supports increased platinum sensitivity in TNBC compared with other breast cancer subtypes and other cytotoxic agents, due to the coupling of platinum induced DNA damage via double strand cross links and deficiencies in BRCA associated DNA repair.

Retrospective analyses support these preclinical findings. A single institution’s results were reported for platinum use in neoadjuvant, adjuvant and advanced disease in TNBC vs. non-TNBC. Neoadjuvant clinical response rates were significantly higher for TNBC compared with non-TNBC (88% vs. 51% respectively, \( p = 0.005 \)).

5-year OS following neoadjuvant/adjuvant chemotherapy was 64% vs. 85% in favour of non-TNBC. Patients with advanced disease with TNBC had a significantly prolonged PFS of 6 months compared with 4 months for non-TNBC (\( p = 0.05 \)). Thus platinum-based chemotherapy was associated with increased pCR but worse OS in early breast cancer, and improved PFS in advanced disease.

TNBC patients with locally advanced disease treated with neoadjuvant platinum and docetaxel were retrospectively identified from a single institution. Of 125 patients, 76 patients also received neoadjuvant adriamycin and cyclophosphamide (AC) and 42 received adjuvant AC. pCR was observed in 42 patients (34%). Within the treatment subgroups, neoadjuvant AC and adjuvant AC were associated with pCR of 40% and 29%, respectively. As shown in other trials, pCR was associated with improved OS (5-year OS: 73% for pCR vs. 49% for non-pCR; \( p < 0.001 \)). Cisplatin appeared superior to carboplatin for OS, however small patient number and multiple variables prevent meaningful conclusions from this observation.

Prospective data is limited to few trials with small patient numbers, with a focus on TNBC patients with BRCA mutation. A neoadjuvant trial of 4 cycles of single agent cisplatin in patients with TNBC and BRCA1 mutation reported pCR in 9 of 10 (90%), 2 of whom received only 2 cycles. The 1 remaining patient had a partial response with residual nodal disease. The same study was extended to a total of 25 women with stage I–III breast cancer with a BCRA1 mutation, regardless of intrinsic molecular subgroup. They received 4 cycles of single agent neoadjuvant cisplatin. Remarkably pCR was observed in 18 patients (72%), suggesting platinum-based chemotherapy is highly effective in patients with BRCA1 associated breast cancer. A neoadjuvant trial of single agent cisplatin in 28 TNBC patients has reported results for 22 patients, of whom 5 achieved a pCR (22%). Lack of a control arm in these small studies makes meaningful interpretation difficult. The addition of carboplatin to paclitaxel was tested specifically in TNBC in the CALGB 40603 neoadjuvant trial. One of the questions asked by this \( 2 \times 2 \) trial design is whether carboplatin adds benefit to paclitaxel and AC.

In the metastatic setting, results are favourable for cisplatin. A single institution phase II study of 126 TNBC patients assessed the addition of weekly cisplatin to metronomic dosing of cyclophosphamide and methotrexate, following prior exposure to an anthracycline and a taxane. This is the only randomized phase II trial to address the issue of the addition of a platinum agent in TNBC. The cisplatin schedule appeared effective and safe, with an ORR of 63% with median time to progression of 13 months. The non cisplatin treated patients had an ORR of 33% with median time to progression of 7 months. Phase III, randomized comparison of first line carboplatin and docetaxel in IHC defined ER-negative, PR negative and HER2-negative metastatic breast cancer is underway in the Triple Negative Breast Cancer Trial (TNT).

Promising early evidence exists for platinum doublets in pre-treated metastatic patients: Carboplatin plus irinotecan in a biologically unselected population of patients with metastatic breast cancer, and carboplatin plus paclitaxel in patients with IHC defined TNBC show response rates of 30% and 57% respectively.

Ixabepilone

Ixabepilone is a new epothilone B analog which binds to tubulin and promotes tubulin polymerization and microtubule stabilization, thereby arresting the cell cycle and inducing tumour cell apoptosis. A prospectively planned subgroup analysis was reported for 187 TNBC patients from the phase III trial of capcetabine with or without ixabepilone, which had 752 patients in total. Benefit was observed from addition of ixabepilone in TNBC with increased response rate from 9% to 27%, and PFS from 2.1 to 4.1 months (HR 0.68, 95% CI 0.50–0.93). A phase II neoadjuvant trial (080 trial) of single agent ixabepilone use in 161 patients revealed pCR in 19% of TNBC patients compared with 8% in non-TNBC.

Novel therapies

From sophisticated molecular biology platforms, novel targeted therapies are evolving. Promising agents include those targeting single strand DNA repair enzyme polyadenosine diphosphate ribose polymerase (PARP), angiogenesis, EGFR and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors.

PARP

PARP is an essential enzyme for base excision repair in single strand DNA. PARP inhibitors induce synthetic lethality in homzygous BRCA deficient cells, whilst sparing cells with preserved BRCA function. Up-regulation of PARP-1 and coexisting impairment of BRCA-mediated DNA repair in TNBC provide the ideal biological platform for PARP inhibition. The PARP enzyme is also involved in initial repair of DNA damage induced by platinum agents and hence, PARP inhibition may be synergistic with cytotoxic DNA damaging agents.

Preclinical data is promising. Cell line data reveal increased sensitivity to PARP inhibition in the majority of TNBC cell lines, regardless of BRCA gene status. Furthermore, the combination of cisplatin and PARP inhibition potentiated cell growth inhibition. PARP inhibitor Phase I and II clinical trial efficacy and safety data are compelling, specifically for orally active Olaparib and parenterally active BSI-201.

A phase I trial of single agent olaparib in refractory cancer patients with various tumour types, enriched with patients with BRCA1/2 mutations, showed olaparib to be active and well tolerated. Whilst not all BRCA mutation carriers responded, all the objective responses were restricted to BRCA mutation carriers, with either breast, ovarian or prostate cancer. A phase II study of single agent olaparib in refractory metastatic breast cancer patients with BRCA1/2 mutation showed single agent response rate of 38%. About 50% of the patients had TNBC, with a similarly high response rate.
Phase I trials reveal efficacy and limited toxicity of BSI-201 in heavily pre-treated patients with various tumour types, when used alone or in combination with chemotherapy.\textsuperscript{62,63} A randomized Phase II study compared carboplatin and gemcitabine with or without BSI-201 in patients with metastatic TNBC who had received between 0 and 2 prior lines of chemotherapy for metastatic disease.\textsuperscript{64} More than 50% of patients had received an adjuvant anthracycline and taxane. Results show significant improvement in overall response rate (48% vs. 16%, \(p = 0.002\)), median PFS (6.9 vs. 3.3 months, \(p < 0.0001\)) and median OS (9.2 vs. 5.7 months, \(p = 0.0005\)) for the BSI-201 containing arm, with no additional toxicity. A registration Phase III randomized trial has commenced in North America testing carboplatin and gemcitabine with or without BSI-201.\textsuperscript{65}

PARP inhibition alone is unlikely to be adequate and combination approaches will be required. Further feasibility and efficacy trials are necessary for PARP inhibitors to move to the adjuvant setting.

**Angiogenesis**

TNBC is associated with abnormal microvascular proliferation. Histological examination demonstrates glomeruloid microvascular proliferation, which correlates with a poor prognosis.\textsuperscript{66} The critical role of angiogenesis may prove a valuable interventional target. Targeted use of antiangiogenic agent bevacizumab was addressed in E2100. This Phase III study assessed bevacizumab plus paclitaxel vs. paclitaxel alone as first line cytotoxic therapy in 722 patients with metastatic breast cancer, the majority of whom (>90%) were HER2-negative. 233 patients had TNBC. Both investigator results and subsequent independent review confirm significant incremental benefit from the combination for both PFS and objective response rate in HER2-negative patients, without OS benefit (HR for PFS by independent review: 0.48, 95% CI 0.385–0.607, \(p < 0.0001\)). Subgroup analysis suggested greater PFS benefit within the TNBC cohort (HR 0.49, 95% CI 0.34–0.70) compared with the non-TNBC [HR 0.57, 95% CI 0.44–0.75].\textsuperscript{67,68}

In a single arm phase II trial, 51 patients with early TNBC were treated with neoadjuvant cisplatin and bevacizumab. Of 46 patients evaluable for response, 7 (15%) had pCR.\textsuperscript{69} CALGB 40603 will explore bevacizumab in TNBC in combination with neoadjuvant chemotherapy.\textsuperscript{53} The BEATRICE study is a phase III open label study assessing the value of adding 1 year of bevacizumab to standard adjuvant chemotherapy in patients with early stage, node positive or negative, centrally confirmed TNBC.\textsuperscript{70}

The multikinase vascular endothelial growth factor inhibitor sunitinib was associated with single agent response rate of 11% in 64 metastatic breast cancer patients previously treated with an anthracycline and a taxane.\textsuperscript{71} Among the subset of patients with triple negative tumours, the response rate was 15% (3 responses in 20 patients). Further results are awaited from a study of single agent sunitinib vs. standard of care in advanced TNBC patients, pretreated with anthracycline and a taxane.\textsuperscript{72}

**EGFR**

TNBC is frequently associated with overexpression of EGFR. However, EGFR overexpression in TNBC has so far not correlated with meaningful clinical benefit from EGFR blocking agents. Certainly, the impressive efficacy of anti-HER2 agents in HER2 overexpressing breast cancer has not been seen with blockade of EGFR in the presence of EGFR overexpression. Results from 2 recent trials have been disappointing. The TBCRC001 multicentre, phase II trial compared cetuximab alone with cetuximab plus carboplatin in pre-treated patients with metastatic TNBC.\textsuperscript{73} Of 102 patients, single agent cetuximab showed minimal activity, with ORR of 6%. The combination showed response of 18%, which may be theorized as predominant activity of the carboplatin alone. Cetuximab has also been assessed in metastatic disease with carboplatin and irinotecan. Whilst addition of cetuximab increased RR in the TNBC patients from 30% with chemotherapy alone to 49%, this translated to neither PFS nor OS benefit.\textsuperscript{74}

**TRAIL**

Tumour survival relies on mechanisms to bypass normal cellular apoptotic pathways. The extrinsic apoptotic pathway requires binding of the soluble ligand TRAIL to Death Receptor (DR) 4 or 5. Clinically, DR4 and 5 may be activated by recombinant TRAIL or agonist monoclonal antibodies. Appealing aspects of TRAIL-induced apoptosis are selective targeting of malignant cells whilst sparing normal cells and synergism when TRAIL-inducing agents are used in combination with chemotherapy.\textsuperscript{75,76}

Early clinical trials in patients with refractory solid tumours reveal TRAIL agonist efficacy as single agent and in combination therapy.\textsuperscript{77,78} Preclinical cell line and animal model results suggest that whilst the majority of breast cancers are resistant, the triple negative subgroup may be particularly sensitive.\textsuperscript{74,79,80} Clinical data with use of TRAIL Targeting therapies specifically in breast cancer is currently lacking.

**Refined therapy for TNBC**

Vast preclinical research is underway to identify and refine potential treatment targets in this aggressive breast cancer subgroup. Studies should investigate the role of specific drugs in predefined subtypes with high likelihood of response based on biological rationale. Adequately powered, prospective clinical trials in the TNBC population will further define the optimal choice and scheduling of chemotherapy and novel agents. Several trial results are awaited with interest. See Table 3.

**Predictive tools**

Not all patients with TNBC will respond equally to therapy. Choice of therapy would ideally be guided by identification of drug targets and/or surrogate predictive biomarkers. Such an approach would impart a degree of pre-treatment certainty of benefit. Factors impacting on efficacy will reflect underlying biological diversity, particularly which pathways and pathway cross talk are dominant for tumour survival and proliferation. TNBC is marked by a high rates of dysfunctional DNA repair, BRCA mutation or dysfunction, p53 mutation, aberrant apoptosis, dysregulated angiogenesis, variable expression of immune response genes and high proliferation. Such features may evolve as predictive of specific treatment benefit. Other hypotheses for potential predictive tools, not restricted to TNBC, include overexpression of the anthracycline drug target, topoisomerase II alpha protein, in predicting anthracycline benefit, or analysis of the p53 pathway in predicting p53-independent taxane activity.\textsuperscript{45,83}

**DNA damage**

Dysfunctional DNA repair in TNBC, reflected in high genomic instability, may render TNBC particular sensitivity to DNA damaging therapy. Tools to assess tumour capacity for DNA repair may aid choice of therapy. Evaluation of baseline DNA damage may identify patients most likely to benefit from DNA targeted therapy, such as anthracyclines, platinum and PARP inhibitors, whilst...
treatment associated DNA damage may reflect efficacy. The Comet assay, an example of such a tool, uses single-cell gel electrophoresis to assess DNA breaks and break frequency by the appearance and intensity of a comet. See Fig. 2. The Comet assay may be combined with fluorescence in situ hybridisation, using labelled probes to particular DNA sequences for analysis at an even finer level of resolution.

A DNA repair profile model, based on 4 genes, was identified and validated as a prognostic tool on TMA created from 143 archived TNBC excision biopsies. The high risk group identified by the profile had a higher recurrence risk and a shorter time to recurrence. Similarly, a gene expression signature from patients with familial BRCA1 mutated breast cancer was applied to 12 patients with sporadic, locally advanced TNBC to search for ‘BRCAness’ and sensitivity to neoadjuvant anthracycline. The BRCA1 gene expression pattern correlated with pCR to anthracycline therapy. A panel of 3 genes was differentially expressed in the sensitive vs. resistant tumours.

**BRCA/BRCAness**

BRCA1 mutation/dysfunction may be not only a prognostic marker, but also a predictive marker of response to chemotherapy. Preclinical studies and exploratory clinical analyses support increased benefit from DNA damaging chemotherapy, namely anthracyclines and platinums, in the presence of BRCA1 dysfunction. Data for correlation of BRCA1 status and taxanes is limited and conflicting. It is tempting to apply BRCA1 findings to TNBC, but tests for ‘BRCAness’, functional assays of the BRCA pathway as mentioned above, would be required to identify which patients. A small study of 15 patients with BRCA1-positive metastatic breast cancer assessed efficacy and safety of single agent cisplatin. Eleven patients had received prior chemotherapy for advanced disease. Of note, 10 of the 15 patients had TNBC whilst 5 were positive for ER or PR. A remarkable response rate of 72% was reported, with 7 (46%) CR and 2 (26%) PR. Further evaluation is required to explore the potential predictive role of BRCA status.

**Predictive gene signatures**

It may be that a single biomarker is too limited in predicting treatment benefit. To incorporate the diversity of disease biology, a predictive panel or signature may be required. Results from predictive gene expression profiling are promising. Interestingly, one study comparing response rates to neoadjuvant chemotherapy between breast cancer subgroups showed that, despite equivalent pCR rates between the basal-like and HER2 subtypes, there was no overlap in the genes associated with pCR for the two groups. This suggests diverse underlying mechanisms of chemosensitivity in the different molecular subtypes and thus, that a therapy pCR predictive signature may be subtype specific. Conversely, a predictive signature may be regimen specific, for example, recent tumour gene

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**Table 3**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control arm</th>
<th>Intervention arm</th>
<th>Primary endpoint</th>
<th>Sample size</th>
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<tr>
<td>CALGB 40603</td>
<td>Paclitaxel → AC</td>
<td>Paclitaxel → AC: + carboplatin + bevacizumab</td>
<td>pCR</td>
<td>362</td>
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<tr>
<td><strong>Adjuvant</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>BEATRICE</td>
<td>Adjuvant chemotherapy</td>
<td>Adjuvant chemotherapy: + bevacizumab</td>
<td>DFS</td>
<td>2530</td>
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<tr>
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<td>AC → ixabepolone</td>
<td>DFS</td>
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<tr>
<td>PACS</td>
<td>FEC → docetaxel</td>
<td>FEC → ixabepolone</td>
<td>DFS</td>
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<tr>
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<td></td>
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<td>OS</td>
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</table>

AC: doxorubicin/cyclophosphamide; DFS: disease-free survival; FEC: 5-fluorouracil/epirubicin/cyclophosphamide; ORR: objective response rate; OS: overall survival; pCR: pathological complete remission.

* PACS aims to recruit 2500 patients in total, 1250 of whom will have TNBC.

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**Fig. 2.** The Comet assay for detection of DNA damage. (a) Normal cells. (b) Cancer cells with DNA damage. The Comet assay requires single cells from tissue or from cell cultures. Cells are embedded in an agarose gel on a slide, exposed to a buffering agent to promote DNA uncoiling and exposed to an electric field. Negatively charged segments of damaged DNA migrate down the electric field away from the intact DNA (the “head”) and generate a “comet” like image (the “tail”). After fluorescent staining, it is possible to measure a variety of parameters, including “tail length” which correlates with size of DNA fragments, “tail intensity” which correlates with DNA break frequency and “tail moment” which combines both the information of tail length and tail intensity. The technique is rapid, sensitive and requires a small number of cells. It allows evaluation of DNA damage and repair at the single-cell level. It may predict patients with particular sensitivity to DNA damaging therapy.
panels showed potential in predicting benefit from FEC or docetaxel plus epirubicin from the EORTC 10994/BIG 001 clinical trial. Compelling results for chemotherapy prediction by a stromal signature is also reported – anthracycline benefit prediction by a 50–gene invito derived stroma-related signature (metagene).

Promising results have recently been reported for a 50–gene breast cancer subtype predictor (PAM50) developed using microarray and quantitative reverse transcriptase polymerase chain reaction. This can be performed on formalin-fixed paraffin embedded tissue. In addition to accurately defining intrinsic subtypes, PAM50 has been shown to add significantly to standard parameters in predicting prognosis and response to neoadjuvant therapy.

Clinical trials is critical. Currently, in the absence of reliable surrogate markers such as triple negativity in breast cancer in clinical practice and across predictive tools, may improve outcomes for TNBC patients. A minority of patients have highly chemosensitive disease with excellent outcome, however tools to prospectively identify these patients and guide chemotherapy agent selection are lacking. Current use of chemotherapy derives from retrospective and/or small studies, or presumption of benefit based on trials in which breast cancer patients were unselected for biological subtype. Biological rationale for efficacy from anthracyclines, taxanes and platinum are within TNBC awaits confirmation in prospective clinical trials. Most TNBC patients have chemoresistant rampant disease, for whom novel targeted therapies, particularly the very promising PARP inhibitors, will hopefully divert the natural course of this disease. Implementation of predictive biomarkers, particularly tools which assess DNA damage and BRCA pathway function, will further refine optimal management.

**Conflict of interest statement**

None declared.

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**References**


