Targeted Therapies in Triple-Negative Breast Cancer

Frederik Marmé, Andreas Schneeweiss

Universitäts-Frauenklinik, University of Heidelberg, Germany;
National Centre for Tumour Diseases, University of Heidelberg, Germany

Introduction

Triple-negative breast cancer (TNBC) constitutes a heterogeneous group of diseases grouped together because of a shared phenotype, which are merely defined by the lack of expression of the oestrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2). Thus, in contrast to other subtypes, TNBC is not defined by shared phenotypic features but only by their absence.

Disease dynamics, risk of recurrence and patterns of metastasis in TNBC differ from other subtypes. Despite a good response to chemotherapy in primary breast cancer (PBC), TNBCs are associated with a 4-fold increased risk of distant metastases and a significantly shorter overall survival [1–6]. The peak of recurrence risk can be observed early on, within 2–3 years after initial diagnosis, reflecting a more aggressive course of disease [3, 7, 8].

A good response to neoadjuvant chemotherapy as defined by a pathological complete remission (pCR) is strongly correlated with very favourable prognosis [9, 10]. Thus, the unfavourable prognosis of TNBCs is predominantly determined by patients who respond poorly to (neo)adjuvant chemotherapy. Patients not achieving a pCR (about 60% of TNBC patients) have a 5-year disease-free survival (DFS) of less than 50% [10]. In order to improve prognosis for those patients not responding to chemotherapy, novel targeted approaches are urgently needed.

Patients with TNBC develop pulmonary, hepatic and cerebral metastases more frequently and bone, lymphatic or soft tissue metastases less frequently than luminal subtypes [11, 12].

However, as mentioned above, TNBC represents a heterogeneous group and there are some histologically defined subtypes, such as classical medullary, apocrine and adenoid-cystic carcinomas, that are typically triple-negative but have a favourable prognosis even without adjuvant chemotherapy [13, 14].

A recent study of TNBCs has revealed at least 6 distinct subtypes defined by their gene expression profiles [15]. These subtypes differ in important biological pathways and prognosis. The subtypes identified were termed basal-like 1 and 2, immunomodulatory, classic, metaplastic, basal-like 3, and HER2-enriched.
Platinum Salts

It might appear strange to comment on a chemotherapeutic agent in a review addressing targeted therapies for TNBC. However, the mode of action of platinum salts at least in BRCA-deficient tumours is similar to a targeted agent. The strong interest in platinum-based therapies in TNBC is based on phenotypic similarities between BRCA1-associated breast cancer and triple-negative disease or more precisely the basal-like subtype. Roughly 80% of BRCA1-associated tumours are basal like. However, the majority of basal-like tumours are not BRCA associated but sporadic. The shared phenotype has led to the speculation that sporadic basal-like tumours might also share defects in homologous recombination (HR) with their BRCA-associated counterparts, albeit caused by different mechanisms like, for example, BRCA methylation and might, therefore, have a similar sensitivity to platinum salts. Platinum-based chemotherapy leads to intra- and inter-strand cross-links, which result in double-strand breaks during replication. These require HR as an error-free DNA-repair mechanism. If a cells harbours HR defects, error-prone compensatory repair mechanisms step in and lead to a high degree of genomic instability, finally leading to the death of the tumour cell. Preclinical data pointed to an extraordinary sensitivity of BRCA-associated breast and ovarian cancers to platinum agents. However, it took a long time until randomized trials provided first evidence that at least a subgroup of TNBC patients might specifically benefit from platinum-based chemotherapy. Several studies in unselected TNBCs revealed discouraging results. For example, the BALI-1 study, a randomized phase II trial that mainly randomized first- and second-line metastatic patients with TNBC to either cisplatin or the combination of cisplatin and cetuximab only yielded an objective response rate (ORR) of 10% for the monotherapy with a median progression-free survival (PFS) of 1.5 months [17]. A further randomized phase II trial, the TBCRC001 study, demonstrated an ORR of 17% for the combination of carboplatin and cetuximab [18]. The TBCRC009, a single-arm phase II trial (n = 86) demonstrated an ORR of 25.6% for carboplatin in unselected first- and second-line TNBCs, which is comparable to responses that can be expected to other chemotherapeutic agents [19]. However, in an exploratory analysis, the ORR was considerably higher in BRCA-associated tumours than in sporadic TNBCs (54.5 vs. 19.7%). This did not, however, result in a prolonged PFS or OS in this small study.

None of these data suggest that there is a superior efficacy of platinum agents in unselected TNBCs. The largest and thus far only trial directly comparing a platinum-based therapy to a taxane in metastatic breast cancer (MBC) has just been presented [20]. The TNT trial randomized 376 patients with metastatic TNBC to either carboplatin or docetaxel. Patients were encouraged to cross over to the alternative monotherapy upon progression if appropriate. There were no significant differences in terms of ORR, PFS and OS during the randomized phase or the cross-over phase in the overall study population. However, an exploratory analysis revealed a significant benefit from carboplatin over docetaxel in BRCA1/2 mutation carriers, with an ORR of 68% versus 33% and a PFS of 6.8 months versus 4.8 months. A test for interaction between BRCA status and therapy was positive. Further preliminary data from the TNT trial suggest that docetaxel might be superior to carboplatin in the non-basal-like subgroup defined by PAM50 [20]. Although these data do not suggest a greater efficacy of platinum agents in unselected TNBCs, they do point to a role in BRCA-associated breast cancer. A small prospective single-arm phase II trial including 20 BRCA1 mutation carriers with MBC who were treated with single-agent cisplatin (75 mg/m² q3w) demonstrated an ORR of 80% with a PFS of 12 months [21].

Two prospective randomized neoadjuvant trials in TNBC, the GeparSixto trial and the CALGB 40603 trial reported an increase in pCR rate (ypT0/is ypN0) of 10.5% and 13% through the addition of carboplatin [22, 23]. Preliminary data from the GeparSixto trial suggest that this gain in pCR rate is mainly driven by BRCA-associated TNBCs, for which the addition of carboplatin increased the pCR rate by 23% [24]. It is still unclear if and how this increase in pCR rate will impact survival, but the data add to the evidence of a specific benefit from platinum agents in BRCA-associated TNBCs. The current AGO guidelines breast cancer version 2015.1D support the use of platinum agents at least in BRCA-associated TNBC (and patients with a positive family history of breast or ovarian cancer) with a single (+) (www.ago-online.de).

PARP Inhibitors

HR represents an important error-free DNA repair mechanism for double-strand breaks. In a process involving BRCA1/2 the homologous sequence of the sister chromatid is used to precisely repair the double-strand break. In BRCA-associated tumours the non-mutated BRCA1/2 allele is inactivated, leading to the accumulation of double-strand breaks and genomic instability. Inhibition of base excision repair (BER) in such cells leads to the accumulation of double-strand breaks during replication, which would usually rely on BRCA-mediated HR. Poly(ADP-ribose) polymerase (PARP) is an enzyme centrally involved in BER by recruiting the
BER machinery to single-strand breaks. Inhibiting PARP in HR-deficient cells leads to the accumulation of double-strand breaks, genomic instability and specific synthetic lethality [25].

Again, phenotypic similarities between TNBCs as a surrogate for the basal-like subtype and BRCA-associated breast cancers have led to the strategy of selecting patients for PARP inhibition on the basis of their TNBC phenotype [26]. Other investigators, however, have chosen to restrict the development of PARP inhibitors to BRCA-associated breast and other solid cancer types, which leaves a considerably smaller target population. Currently, several PARP inhibitors such as olaparib, veliparib, rucaparib, niraparib, talazoparib (BMN673) and others are undergoing clinical development. Olaparib was the first PARP inhibitor to be granted regulatory approval for recurrent high-grade serous ovarian cancer by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). Olaparib was first developed in a single-arm phase II study recruiting patients with BRCA-associated breast cancer in 2 consecutive cohorts treated with 100 mg twice daily (bid) and 400 mg bid single-agent olaparib, respectively. Results of this trial demonstrated a promising dose-dependent ORR of 22% (100 mg bid) and 41% (400 mg bid) with a median PFS of 5.7 months for the higher dose [27]. A parallel trial in BRCA-associated ovarian cancer showed comparable results [28].

Gelmeti et al. [29] studied the efficacy of olaparib in unselected TNBCs not restricted to BRCA-associated breast cancer and unselected serous ovarian cancer. Their study included 26 patients with TNBC but was unable to demonstrate any responses as defined by RECIST (Response Evaluation Criteria in Solid Tumors). In contrast to an ORR of 41% (BRCA mutated) and 24% (BRCA wild type) seen in the ovarian cancer cohort, no confirmed responses were seen among the 26 breast cancer patients. A recent study including several BRCA-associated solid tumours demonstrated a discouraging ORR of only 12.9% with a PFS of only 3.7 months in the 62 BRCA-associated breast cancer patients included. These patients were heavily pre-treated with a mean of 4.6 prior regimens for advanced disease. Two thirds had also received prior platinum-based therapy. The ORR seemed to be higher in breast cancer patients without prior platinum chemotherapy (20% vs. 9.5%). However, the 193 BRCA-associated ovarian cancers included in this study had all received prior platinum chemotherapy and were considered to be platinum resistant or not suitable for platinum therapy, yet still had an ORR of 31% and a PFS of 7.0 months. Overall, these data suggest that PARP inhibition by olaparib is more effective in (BRCA-associated) ovarian cancer than in BRCA-associated breast cancer. A cross-trial comparison might suggest a superior efficacy of olaparib in the trial by Tutt et al. [27], which demonstrated an ORR of 41% in the 400 mg bid cohort. Whether this is due to a lesser extent of pre-treatment and fewer patients with prior platinum chemotherapy is a matter of speculation. Trials attempting to combine olaparib with paclitaxel failed to achieve a meaningful dose intensity of paclitaxel because of high rates of neutropenia despite G-CSF (granulocyte colony-stimulating factor) prophylaxis.

As part of the I-Spy 2 trial the combination of carboplatin and veliparib added to weekly paclitaxel and followed by doxorubicin/cyclophosphamide led to a doubling of the pCR rate in the triple-negative study population (from 26% to 52%). Trial statistics in a phase III trial predicted a probability of success of 90% for this combination [30]. Table 1 summarizes current phase III trials investigating PARP inhibitors in breast cancer.

Several mechanisms of PARP inhibitor resistance in BRCA-associated tumours have been proposed. These include genetic reversal of truncating mutations, stabilisation of mutated BRCA proteins, e.g. by HSP90 (possibly targetable by HSP90 inhibitors), loss of 53BP1, hypomorphic BRCA function and drug efflux pumps [31]. Several strategies to overcome these resistance mechanisms are currently under investigation. The exploration of these mechanisms as potential predictive factors is also warranted.

### Anti-Angiogenesis

TNBC is associated with a significantly higher expression and more frequent amplification of vascular endothelial growth factor
(VEGF)-A [32, 33]. This has led to the hypothesis of a specifically higher activity of anti-angiogenic drugs in TNBC. Several anti-angiogenic agents are currently under development. Bevacizumab, a monoclonal anti-VEGF-A antibody is the only drug currently approved for the treatment of MBC by the EMEA, but not by the FDA.

In the pivotal open-label randomized phase III trial, E2100, in first-line unselected patients, the addition of bevacizumab to weekly paclitaxel increased median PFS from 5.9 to 11.8 months (hazard ratio 0.6, p < 0.001) and doubled the response rate (49.2% vs. 25.2%, p < 0.001). However there was no significant improvement in OS [34, 35].

Two further first-line phase III studies (AVADO and RIBBON-1) confirmed the benefit of the addition of bevacizumab to first-line chemotherapy, although to a lesser extent [36–38]. The efficacy of bevacizumab in addition to weekly paclitaxel could further be confirmed by similar results observed for the combination in the TURANDOT study (PFS 11 months, ORR 44%) and CALGB 40502 study (PFS 10.6 months) [39, 40].

However, neither of these individual randomized trials nor a joint analysis was able to demonstrate a significant OS benefit in unselected patients. A trend towards an improved OS for the TNBC cohort (17.9 vs. 12.6 months, p = 0.0534), in addition to a significant PFS benefit (6.0 vs. 2.7 months; hazard ratio 0.45; p = 0.0006), was observed only in an explorative subgroup analysis of the randomized phase III second-line trial, RIBBON-2 [41]. However, this can at best be regarded as a hypothesis-generating retrospective analysis due to small number of patients (n = 159) and statistical considerations. Bevacizumab is not approved for second-line treatment.

The important question addressing the hypothesis of a specific bevacizumab benefit in TNBCs was addressed in a joint subgroup analysis of all TNBCs within the 3 randomized phase III first-line trials. This analysis included 621 patients with TNBC from these trials and confirmed the increased ORR (42% vs. 23%) and PFS (8.1 vs. 5.4 months; hazard ratio 0.63; p < 0.0001), although without a trend for an improved OS (18.9 vs. 17.5 months; hazard ratio 0.96; ns) [42].

The joint analysis only found a significantly improved OS for triple-negative patients who had previously received a taxane-containing adjuvant regimen (25.6 vs. 15.0 months; hazard ratio 0.61, 95% confidence interval 0.4–0.94). However, this was only an explorative subgroup analysis of a subgroup analysis.

Data from a large adjuvant phase III trial exclusively run in TNBC, the BEATRICE trial (n = 2,591), did not demonstrate an improvement in DFS or OS for the addition of bevacizumab to adjuvant chemotherapy followed by bevacizumab maintenance therapy [43].

Data from neoadjuvant studies are inconsistent with respect to a specific benefit from bevacizumab in TNBCs, some demonstrating a small increase in pCR rates in ER-positive cases, others in the TNBC population [22, 44, 45].

In summary, the effect of bevacizumab in TNBCs is similar to that seen in unselected patients. However, because of limited therapeutic options and frequent aggressive disease dynamics, bevacizumab might play a special role in the treatment of metastatic TNBC. Higher response rates and longer PFS can more effectively counteract and control existing or threatening symptoms without the need for polychemotherapy with all its toxicities.

One of the main drawbacks for anti-angiogenic therapies including bevacizumab is the lack of a clinically validated predictive biomarker. Triple-negative status does not play a role in predicting benefit in this respect. Currently, a prospective randomized trial, the Meridian trial (NCT01663727), is investigating the role of serum VEGF-A as a potential factor for patient selection for bevacizumab.

In addition to monoclonal antibodies, a series of tyrosine kinase inhibitors (TKIs) against pro-angiogenic kinases like receptors for VEGF and PDGF have been developed, e.g. sunitinib, sorafenib and pazopanib. Because of the increased off-target effects of these TKIs, combination with chemotherapeutic agents has proven difficult. Their efficacy as monotherapy in MBC is limited with ORRs ranging from 0% to 11% [46–50].

Sunitinib and sorafenib have been developed in phase Ib/III programmes. Sunitinib was unable to provide any benefit either as monotherapy compared to capcitabine or in combination with chemotheraphy [49, 51–53]. There are no specific data for triple-negative patients from these trials. Sorafenib demonstrated activity in a phase Ib study in combination with either capcitabine or gemcitabine, although at the cost of a high rate of palmar-plantar erythema (45% grade 3) [54]. In the light of their modest activity but considerable toxicity, it is unlikely that anti-angiogenic TKIs will play an important role in the treatment of MBC, regardless of subtype.

**Epidermal Growth Factor Receptor**

Overexpression of epidermal growth factor receptor (EGFR) can be observed in more than 50% of TNBCs and is correlated with a poor prognosis [55, 56]. This observation prompted a series of clinical trials incorporating anti-EGFR directed therapies, such as cetuximab and lapatinib.

The TBCRC001 trial investigated the activity of cetuximab, a monoclonal anti-EGFR antibody as a single agent and in combination with carboplatin. Single-agent cetuximab demonstrated a disappointing ORR of 6% with a clinical benefit rate of only 10%. The combination with carboplatin resulted in an ORR of 17%. The median time to progression for single-agent cetuximab was only 1.7 months and 2.6 months for the combination. However, despite these disappointing results, 4 patients (2 in the monotherapy and 2 in the combination arm) in the trials had long-lasting responses of more than 12 months (2 even over 2.5 years) [18]. Results from serial biopsies available for 18 patients within the trial showed that 81% of the patients demonstrated EGFR-pathway activation; however, this could only be inhibited by cetuximab in 5 of the patients (38%). Clinical benefit was restricted to these patients [18]. Thus, apart from EGFR overexpression, downstream activation of the...
pathway might play an important role. The largest published randomized study so far, the BALI-1 trial including 173 patients with metastatic TNBC, 73% in a first-line setting, randomized patients to either a combination of cetuximab and cisplatin or cisplatin alone. There was a significant benefit in terms of PFS for the combination (3.7 vs. 1.5 months; hazard ratio 0.675; \( p = 0.032 \)) and a non-significant improvement in response rate (20% vs. 10.3%; \( p = 0.11 \)) and OS (12.9 vs. 9.4 months; hazard ratio 0.82; \( p = 0.31 \)). A possible impact of the 53% of patients crossing over to cetuximab containing regimens at progression remains a matter of speculation [17].

In addition, there are no data on activation of EGFR signalling (e.g. EGFR expression, etc.) or KRAS status and downstream activation of the pathway, which like in colorectal cancer could impact the efficacy of cetuximab in MBC. Currently, panitumumab, a human monoclonal anti-EGFR antibody is under investigation in the efficacy of cetuximab in MBC. Currently, panitumumab, a human monoclonal anti-EGFR antibody is under investigation in the treatment of TNBC. Immunotherapeutic approaches may offer one strategy to circumvent these problems. Table 2 gives a summary of results from selected trials.

**Further Experimental Therapeutic Targets**

**Immune Checkpoint Inhibitors**

Rapid progression and the development of resistance pose the main challenges in the treatment of TNBC. Immunotherapeutic approaches may offer one strategy to circumvent these problems. TNBCs harbour more non-synonymous mutations compared to luminal-type breast cancers and more tumour-infiltrating lymphocytes, which in TNBC have a strong predictive and prognostic meaning. The non-synonymous mutations may act as neoantigens that could principally be recognized by the host immune system. The ability of neoantigens to elicit an immune response is dependent on their ability to form stable complexes with major histocompatibility complex (MHC) I molecules and therefore to be presented and recognized by specific T-cell receptors. Whereas the mere presence of non-synonymous mutations is not prognostic in several types of cancers, immunogenic neoantigens as predicted by validated algorithms are independent prognostic factors [61]. Tumours exploit several mechanisms to evade the recognition or destruction by the host immune system. Amongst them is the downregulation of MHC I molecules. Further, CTLA-4 and PD-1 (programmed death receptor 1) are 2 key cell-surface receptors that dampen T-cell response by triggering inhibitory pathways should be more precisely defined by clinically validated predictive factors. In many cases constitutive EGFR-pathway activation seems to be at least partially due to downstream activation of the pathway, e.g. through KRAS amplification/mutation or CRYAB expression [18, 59]. Successful targeted therapies in these cases will require different strategies.

**Table 2. Results from selected trials with EGFR-targeted therapies including TNBCs**

<table>
<thead>
<tr>
<th>Study [Ref]</th>
<th>Population</th>
<th>Regimen</th>
<th>n</th>
<th>ORR</th>
<th>OR</th>
<th>PFS/ hr</th>
<th>OS hr</th>
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<tbody>
<tr>
<td>BALI-1 [17]</td>
<td>mTNBC</td>
<td>cisplatin</td>
<td>58</td>
<td>10.3</td>
<td>1.5</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>TBCRC001 [18]</td>
<td>mTNBC</td>
<td>cisplatin + cetuximab</td>
<td>115</td>
<td>2.13</td>
<td>0.67</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mTNBC</td>
<td>cetuximab</td>
<td>17</td>
<td>2.13</td>
<td>0.82</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mTNBC</td>
<td>PD-RTK-183 + cetuximab</td>
<td>25/31</td>
<td>2.6*</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>USON 225200 [60]</td>
<td>MBC</td>
<td>irinotecan/cisplatin</td>
<td>75</td>
<td>31</td>
<td>4.6</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>irinotecan/cisplatin/cetuximab</td>
<td>79</td>
<td>38</td>
<td>4.8</td>
<td>12.6</td>
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</tr>
<tr>
<td>EGF30001 [57,58]</td>
<td>HER2+ MBC</td>
<td>paclitaxel</td>
<td>37</td>
<td>5.0</td>
<td>19.2</td>
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<tr>
<td>EGF30001 [57,58]</td>
<td>HER2- MBC</td>
<td>paclitaxel/lapatinib</td>
<td>49</td>
<td>3.0</td>
<td>8.1</td>
<td>12.3</td>
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<tr>
<td></td>
<td>mTNBC</td>
<td>paclitaxel</td>
<td>204</td>
<td>23.5</td>
<td>5.4</td>
<td>20.3</td>
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<tr>
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<td>paclitaxel/lapatinib</td>
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<td>1.4</td>
<td>5.4</td>
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<td>paclitaxel</td>
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<td>–</td>
<td>4.6</td>
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</tr>
<tr>
<td></td>
<td>mTNBC</td>
<td>paclitaxel/lapatinib</td>
<td>71</td>
<td>–</td>
<td>4.8</td>
<td>0.26</td>
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</table>

EGFR = epidermal growth factor receptor, TNBC = triple-negative breast cancer, mTNBC = metastatic TNBC, ORR = objective response rate, PFS = progression-free survival, hr = hazard ratio, TTP = time to progression, MBC = metastatic breast cancer.
when bound by their ligands. For the PD-1 pathway, ligand expression (PD-L1) frequently occurs on tumours cells, which directly leads to death of reactive T cells. Current therapeutic strategies to block these immune checkpoints mostly engage CTLA-4, PD-1 and PD-L1. Ipilimumab, a monoclonal anti-CTLA-4 antibody, nivolumab and pembrolizumab (monoclonal antibodies against PD-1) have been approved for the treatment of several solid tumours and have led to prolonged responses. First data to suggest that such immune checkpoint inhibition strategies might be successful in TNBC were presented at the San Antonio Breast Cancer Symposium in December 2014. Pembrolizumab demonstrated an ORR of 18.5% in a phase I b study (n = 32) in PD-L1-positive TNBCs, most of which were heavily pretreated. Response rates were independent of the number of prior lines of therapy. Amongst those patients responding, responses were long lasting, with the median duration of response not reached at the time of presentation [62]. Inhibition of PD-L1 by the monoclonal anti-PD-L1 antibody MPDL3280A in PD-L1-positive TNBCs (n = 12) yielded an ORR of 33% in a phase I a trial, again with long-lasting responses [63]. The duration of response seen in these heavily pretreated patients with metastatic TNBC is extraordinary and encouraging. Immune checkpoint blockade might in fact constitute the most promising of targeted therapies for TNBC currently being investigated.

**Androgen Receptor**

A gene expression study in 99 breast cancer cases, including 41 TNBC, demonstrated that a subgroup of TNBCs was grouped together with ER-positive cases in a cluster analysis. Further investigations revealed that, despite the lack of ER and PgR expression, these cases show an expression of an ER-regulated gene set. This is caused by the expression of the androgen receptor (AR) in these cases. TNBC cell lines with the respective phenotype respond to (neo)adjuvant chemotherapy (NACT), they analysed the residual tumours was associated with poor prognosis in a multivariate analysis (hazard ratio 4.16, p = 0.002). Even though these results have to be validated, ruxolitinib, a JAK2 inhibitor, is currently approved for the treatment of myelofibrosis.

In conclusion, current therapy for TNBC is mainly analogous to unselected MBC, maybe with the exception of platinum in BRCA-associated tumours. There is still no targeted therapy with specific activity in TNBC. However, the role of PARP inhibitors and immunotherapies might lead to future improvements, as might targeted therapies directed against the resistant residual tumour after NACT.

**Disclosure Statement**

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References
