Triple-negative breast cancer—current status and future directions

O. Gluz¹, C. Liedtke², N. Gottschalk³, L. Pusztai⁴, U. Nitz¹ & N. Harbeck³

¹Westdeutsche Studiengruppe GmbH, Mönchengladbach; ²Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Universitätsklinikum Münster, Münster; ³Brustzentrum Köln/Frechen, Uniklinikum Köln, Köln, Germany and ⁴Department of Breast Medical Oncology, MD Anderson Cancer Center, Houston, TX, USA

Received 23 July 2009; revised 24 August 2009; accepted 8 September 2009

Triple-negative breast cancer (TNBC) is defined by a lack of expression of both estrogen and progesterone receptor as well as human epidermal growth factor receptor 2. It is characterized by distinct molecular, histological and clinical features including a particularly unfavorable prognosis despite increased sensitivity to standard cytotoxic chemotherapy regimens. TNBC is highly though not completely concordant with various definitions of basal-like breast cancer (BLBC) defined by high-throughput gene expression analyses. The lack in complete concordance may in part be explained by both BLBC and TNBC comprising entities that in themselves are heterogeneous. Numerous efforts are currently being undertaken to improve prognosis for patients with TNBC. They comprise both optimization of choice and scheduling of common cytotoxic agents (i.e. addition of platinum salts or dose intensification strategies) and introduction of novel agents (i.e. poly-ADP-ribose-polymerase-1 inhibitors, agents targeting the epidermal growth factor receptor, multitargeted kinase inhibitors or antiangiogenic agents).

Key words: basal-like breast cancer, gene expression profiling, molecular heterogeneity, targeted agents, triple-negative breast cancer

introduction

Breast cancer (BC) is the most common female cancer. More than 1 million women worldwide are affected by this diagnosis and ~400 000 patients die due to the disease every year. Implementation of mammography screening as well as improvement of adjuvant systemic treatment and a decrease in hormone replacement therapy use have resulted in a decrease in both BC incidence and particularly mortality in developed countries over the past 5 years [1]; worldwide, however, the incidence of BC is nevertheless increasing.

molecular heterogeneity of BC

definition of triple-negative BC

BC is increasingly recognized as a heterogeneous disease exhibiting substantial differences with regard to biological behavior and requiring distinct therapeutic interventions. Steroid hormone receptors (HR) such as estrogen receptor (ER) and progesterone receptor (PgR) in concert with the oncoprotein ErbB-2/human epidermal growth factor receptor 2 (HER-2) are critical determinants of these BC subtypes. While HR are thought to mirror a good prognosis [2], expression of HER-2 has long been understood as an unfavorable prognostic feature [3]. However, since the introduction of trastuzumab as a potent therapeutic approach in HER-2-positive BC, HER-2 expression is perceived as a favorable predictive rather than negative prognostic factor [4–6].

Triple-negative breast cancer (TNBC) is characterized by a lack of expression of both ER and PgR as well as HER-2. Thus, to date, chemotherapy remains the only possible therapeutic option in the adjuvant or metastatic setting in the TNBC. A recent analysis indicates that TNBC carries a distinct molecular profile when compared with HR-positive BC. Investigating gene expression profiles of 764 patients randomized in the E2197 study, the authors demonstrated that 269 of 371 genes associated with kinase activity, cell division, proliferation, intracellular DNA repair, antiapoptosis, and transcriptional regulation were differentially expressed between both subtypes [7].

definition of basal-like BC

In their pivotal paper, Perou et al. [8] have demonstrated that high-throughout gene expression analysis carries the potential to capture the heterogeneity of the disease and distinguish BC subclasses solely on the basis of differences regarding their gene expression profile. They demonstrated that expression of ER and HER-2 represents two major determinants of BC molecular subgroups. Using unsupervised hierarchical clustering, Sorlie et al. [9] pioneered the establishment of a BC classification system distinguishing five distinct BC molecular subgroups. Luminal A and B subtypes were identified to be largely ER...
positive. In contrast, three groups were characterized by low ER expression and denominated as follows:

- basal-like breast cancer (BLBC) being characterized by lack of expression of ER, PgR, HER-2 (i.e. triple negativity) as well as an increased expression of basal (myoepithelial) cytokeratins (CKs) such as CK5/6 and CK17,
- erbB2-like/HER-2-like BCs showing an increased high expression of genes associated with the erbB2 amplico and
- normal-like BCs sharing molecular features of normal breast tissue. This group has recently been indicated to represent an artifact of having a high percentage of contamination of normal breast tissue in the specimen rather than a distinct BC subtype [10]. However, detailed histological, immunohistochemical, and genomic data will be required to support this hypothesis.

The BLBC/TNBC and erbB2 subtypes in particular have been reproduced in subsequent reports. For instance, Sorlie et al. [9] refined the molecular classification by using an ‘intrinsic gene set’ containing 456 complementary DNA clones in 78 T3/4 tumors, 51 of which were treated on a neoadjuvant basis with doxorubicin monotherapy. Importantly, both basal-like (BL) and HER-2-like subgroups were associated with a significantly decreased survival in comparison to the luminal A subgroup which carried the most favorable prognosis.

The term ‘basal-like’ BC stems from the resemblance of its expression pattern to the one observed among normal basal/myoepithelial cells of the breast which comprise high-molecular-weight basal CKs (CK5/6, CK14, CK17), vimentin, p-cadherin, β-crystalline, caveolins 1 and 2, as well as the epidermal growth factor receptor (EGFR). Consequently, it has been indicated that BLBCs arise from the outer (basal) layer of normal breast ducts (i.e. myoepithelial cells) or perhaps more accurately originate from a stem cell precursor of basal myoepithelial cells. In contrast, luminal cancers may originate from a more differentiated luminal precursor cell [11]. A number of subsequent reports support this hypothesis:

- Increased expression of keratin 14 in contrast to low expression of keratin 18 are characteristics of cells carrying the potential to self-renew and differentiate into both luminal and myoepithelial cells [12].
- BLBCs commonly express an ‘embryonic stem-cell signature’ [13].
- BLBCs exhibit well-established characteristics of epithelial–mesenchymal transition, such as loss of epithelial characteristics and acquisition of a mesenchymal phenotype [14].
- BLBCs frequently express a CD44+/CD24− phenotype which has been associated with a ‘stem-cell’ phenotype [15].

**association between TNBC and BLBC**

Despite the common understanding that BLBC carries unfavorable (and therefore clinically relevant) prognostic features, no widely accepted and clinically usable (i.e. robust, reproducible and standardized) assay is currently available to define BLBC status and there is good but imperfect concordance between the TNBC and BLBC demonstrating that heterogeneity within groups defined by either one of the above classification methods poses a significant limitation to each method.

**concordance between TNBC and BLBC phenotype**

Several studies have demonstrated that BL tumors are not necessarily triple negative (TN). For instance, up to 15%–45% of BLBCs have been shown to express ER [9, 16] and 14% of BLBCs to express HER-2 [16], indicating that not all BLBCs regardless of classification method are TN. Conversely, while 16%–44% of TN cases are negative for all basal markers (CK5/6, CK14, EGFR) [17, 18], 7.3% of non-TNBCs do express these [19]. In later studies 71% of TNBCs were reported to be positive for at least one basal marker (i.e. CK5/6, CK17, CK14, EGFR) [20].

Birnbaum et al. compared the definition of TNBC with a more complex gene expression-based definition of BL status applying a 500-gene set to 172 cases of TNBC; 123 of these were BL (71.5%). Conversely, 160 BL tumors included 37 cases of non-TNBC [21]. A retrospective analysis of the WSG AM 01 high-risk BC trial corroborates these results; in this study, only 33% of 66 TNBCs were clustered as BL by k-clustering of 24 protein expression profiles; importantly, 44% of TNBCs were completely negative for all measured basal markers (i.e. EGFR, CK5 and 17, vimentin, c-kit) [22].

**heterogeneity within TNBC and BLBC**

Scientific efforts have aimed for an identification of (immunohistochemical) markers in conjunction with TNBC status hypothesizing that TNBC is a heterogeneous entity with BLBC representing only one presumable subtype.

For instance, EGFR expression may be found among 57% of BL but only 8% of non-BLBC cases [23]. Nielsen et al. [24] indicated a so-called ‘five-marker method’ (TN and either EGFR or CK5/6 positive), which identified gene expression-based BLBC with a sensitivity of 76% and a specificity of 100%. Conversely, only 85% of TN tumors were truly BL. Recently, in a retrospective analysis of 3744 cases, 17% and 9% stained as TN and BL (by the five-marker method) [25].

Recent evidence indicates that several further divergent pathways may be active within TNBC. So Sparano et al. [26] identified that expression of growth factor receptor-bound protein 7 (a key element in cell signaling, motility and migration) was lower among TNBCs and significantly associated with outcome in multivariate analysis.

In summary, divergences of BL and TNBC may be explained by the existence of two distinct subtypes within the TN phenotype, i.e.

- a gene expression-based BL versus a normal-like subtype (as defined by Sorlie et al. [9]) or
- an immunohistochemistry-based basal marker-positive [27] versus a multiple marker-negative subtype [22].

Most importantly, while the predictive impact of conventional ER, PgR and HER-2 measurements in the clinical setting is relatively clear, the clinical significance of molecular class remains to be determined. Non-BL TNBCs may carry a more favorable prognosis and increased chemotherapy sensitivity
histological presentation of BLBC/TNBC

More than 90% of BLBCs/TNBCs exhibit an invasive ductal histology and high histological grade, present with high mitotic index and carry central necrotic zones and pushing borders as well as a conspicuous lymphocytic infiltrate [28–33]. Additional characteristics of BLBC are frequent metaplastic elements and medullary/atypical medullary features [28,29,34,35]. Recent reports confirm that very aggressive metaplastic tumors are BL by expression analysis [36].

epidemiology and risk factors

The epidemiological risk factors for TNBC compared with non-TNBC appear to differ significantly (Table 1). Overall, the prevalence of TNBC in large unscreened breast cancer patient cohorts is ~11%–20% [17, 25, 33], whereas in selected cohorts of patients with advanced BC or patients of African-American ethnicity, TNBC may be diagnosed among as many as 23%–28% of all patients [37, 39, 52]. The close correlation with African-American ethnicity seems to be independent of an increased frequency of obesity in this patient population or age [53].

diagnostic patterns

Rare scientific data indicate that a reduced incidence of microcalcifications and peritumoral ductal carcinoma in situ (DCIS) represent typical mammographic characteristics comprise [33, 54]. Consistent with its more aggressive biology, this BC subtype very often manifests itself as an interval cancer [i.e. diagnosed between (screening) mammograms] [33, 42, 55]. Furthermore, unilocularity, mass lesion type, smooth mass margin, rim enhancement, persistent enhancement pattern, and very high intratumoral signal intensity on T2-weighted magnetic resonance images are typical features associated with TNBC [56]. Magnetic resonance imaging (MRI) carries a particular potential to predict response to neoadjuvant chemotherapy in TNBC [57, 58]. Furthermore, TN breast tumors show enhanced 2-[fluorine-18]fluoro-2-deoxy-D-glucose (FDG) uptake allowing for detection of TNBC with a high sensitivity by using FDG–positron emission tomography (FDG–PET) [59].

outcome in TNBC

survival

TNBC accounts for a disproportionate number of BC deaths; the majority of studies indicate a negative impact of a TN (on the basis of data of thousands of patients) or BL (defined by a few molecular studies) phenotype on patient prognosis [17, 24, 37, 39, 44, 46–62]. In numerous randomized trials, patients with TN [63, 64] or BL [65] tumors treated by anthracyclines and taxanes experience a significantly decreased survival compared with patients with other tumor types. Importantly, the prognostic effect of TNBC is independent of poor grade, nodal status, tumor size and treatment [44, 63]. The aggressiveness of TNBC is further indicated by the fact that (i) the peak risk of recurrence occurs within the first 3 years after initial treatment of the disease with the majority of deaths occurring in the first 5 years [18, 42] and (ii) after diagnosis of metastatic disease, a significantly shorter survival was observed in both BL [28] and TNBC [42, 45]. Conversely, the risk for late recurrences (i.e. beyond 5 years of diagnosis) is decreased by 50% compared with HR-positive disease [27]. However, differences between TNBC and non-TNBC regarding overall survival (OS) wear off at 10 years of follow-up. Cheang et al. recently hypothesized that the negative impact of TNBC on survival may be affected only by the subgroup of basal tumors within the TNBC group. Using the five-marker method described above, patients with BL TNBC had significantly decreased BC-specific OS compared with patients with the remaining non-basal TNBC; among patients treated by adjuvant anthracycline-based chemotherapy, the addition of basal markers allowed for identification of a subgroup with a significantly increased risk of relapse [25, 66]. These results are in line with other studies [27], particularly in node-negative patients [17].

local and locoregional recurrence

Although the association between TNBC/BLBC and a less favorable prognosis has been clearly established, the effect on risk of local and distant recurrence remains less clear. Several studies have supported a significantly increased rate of visceral versus bone metastasis [47, 67] among patients with TNBC compared with non-TNBC. In the largest report to date, data on 12 858 patients indicate an increased risk for lung (odds ratio (OR) 2.27) and brain (OR 5.32) metastasis as first site of recurrence and lower risk for bone recurrence (OR 0.23) in patients with TNBC [33]. For further details see Table 2.

incidence of central nervous system metastases

Patients with TNBC compared with other subtypes reportedly experience an increased risk of central nervous system metastases (CM) of 6%–46% of those experiencing metastatic spread of disease [75–77]. Similarly, in a single-institution

Table 1. Risk factors for TNBC

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>High parity, young age at the time of first birth, lack of breast-feeding, use of oral contraceptives (in women &lt;40 years old)</td>
<td>[37–40]</td>
</tr>
<tr>
<td>Younger age at diagnosis (i.e. &lt;50 years)</td>
<td>[18,41–43]</td>
</tr>
<tr>
<td>African-American ethnicity</td>
<td>[33,39,44–46]</td>
</tr>
<tr>
<td>Hispanic ethnicity</td>
<td>[47]</td>
</tr>
<tr>
<td>Lower socioeconomic status</td>
<td>[48]</td>
</tr>
<tr>
<td>Increased body weight</td>
<td>[33,49,50]</td>
</tr>
<tr>
<td>Metabolic syndrome (particularly high blood glucose, high triglyceride, or low HDL levels)</td>
<td>[51]</td>
</tr>
</tbody>
</table>

TNBC, triple-negative breast cancer; HDL, high-density lipoprotein.
Table 2. Outcome among patients with TNBC

<table>
<thead>
<tr>
<th>Authors</th>
<th>N overall (TNBC)</th>
<th>Follow-up</th>
<th>Patient characteristics and therapy</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffty et al. [41]</td>
<td>N = 482 (118)</td>
<td>7.9 years</td>
<td>75% node-negative BC: BCT and RT; adjuvant CHT: 69% versus 41.6% TNBC versus non-TNBC</td>
<td>Local control 83% in both cohorts</td>
<td>Increased ipsilateral axillary node relapse and poorer DFS in the TNBC cohort.</td>
</tr>
<tr>
<td>Dent et al. [42]</td>
<td>N = 1601 (118)</td>
<td>8.1 years</td>
<td>TNBC versus other: 54.4% versus 45.6% node positive, mean tumor size 3.0 versus 2.1 cm; adjuvant CHT: TNBC, 48.6% and other, 25.5%</td>
<td>TNBC versus other—LR: 13% versus 12%; DR: 34% versus 20.4%</td>
<td>Shorter time to both LR and DR in TNBC group; relative risk only in the first 3 years; HR 4.0 for visceral metastases and 0.8 for bone metastases in TNBC group versus others [68]; risk of DR but not time to relapse is independent of tumor size and lymph node status [69].</td>
</tr>
<tr>
<td>Freedman et al. [70]</td>
<td>N = 753 (98)</td>
<td>Not reported; database 1990–2006</td>
<td>TNBC versus HR+: 32% versus 16% pT2 tumors, all pT1/2, in all BCT and RT, adjuvant CHT: TNBC versus HER-2+ versus HR+: 64% versus 73% versus 2%</td>
<td>TNBC versus HER-2+ versus HR+: 5-year isolated LR 3.2% versus 4.6% versus 2.3%; 5-year DR 6.5% versus 11.9% versus 3%</td>
<td>Higher incidence of LR and coexistent distant metastases in the TNBC group compared with HR+ (5.3% versus 2.6%, P = 0.05); no significant OS difference.</td>
</tr>
<tr>
<td>Kyndi et al. [71]</td>
<td>N = 1000 (randomized N = 3083) (153)</td>
<td>17 years</td>
<td>Significantly poorer tumor characteristics in TNBC group; all treated by mastectomy ≤RT; adjuvant CMF CHT TNBC versus HR+/-HER-2—: 64% versus 55%</td>
<td>TNBC versus HR+/-HER-2— versus HR—/ HER2+: LR alone—18% versus 13.4% versus 16%; higher rates for DR in the TNBC, addressed to the no-RT group</td>
<td>Decreased OS in the TNBC group, in the treated by RT group association only with LR, not OS or DM; benefit of RT in terms of LR in all groups; no OS benefit in TNBC and HR+/-HER2+ group.</td>
</tr>
<tr>
<td>Rodriguez-Pinilla et al. [67]</td>
<td>N = 227 (27 BLBCs)</td>
<td>8.4 years</td>
<td>Node-negative BC, larger tumor size in the BLBC (23% T2); ~50% treated by adjuvant CMF CHT</td>
<td>BLBC versus other: LR 17.4% versus 5.3%, visceral 13% versus 3.8%, bone 8.2% versus 0%</td>
<td>DFS difference only in the CHT-untreated patients.</td>
</tr>
<tr>
<td>Nguyen et al. [72]</td>
<td>N = 793 (89)</td>
<td>5.8 years</td>
<td>TNBC versus HR+/HER-2— versus HR+/HER2+: N+, 34% versus 26% versus 50%; T1, 84% versus 66% versus 62%; all patients BCT+RT, adjuvant CHT 76% versus 37% versus 66%</td>
<td>TNBC versus HR+/HER-2— versus HR+/ HER2+: 5-year LR—7.4% versus 0.8% versus 8.4%; 5-year DR—19% versus 3.3% versus 16%</td>
<td>In the multivariate analysis only, TNBC and luminal B (HR+/-HER-2+) are significantly associated with DFS.</td>
</tr>
<tr>
<td>Liedtke et al. [47]</td>
<td>N = 1118 (225)</td>
<td>2.9 and 3.8 years (TNBC and non-TNBC, respectively)</td>
<td>All treated by neoadjuvant CHT, BCT more frequent in the TNBC 40.4% versus 30.4%</td>
<td>Relapse sites in TNBC versus non-TNBC—visceral: 74% versus 63%; bone: 13% versus 27%</td>
<td>Decreased OS and DFS (addressed to the non-PCR group of the TNBC), shorter survival after-relapse survival (irrespective of localization), increased risk only in the first 3 years.</td>
</tr>
<tr>
<td>Kaplan and Malmgren [73]</td>
<td>N = 1550 (183)</td>
<td>4.24 years</td>
<td>Higher TNM stage in the TNBC versus HR+/HER2— (stage III 17% versus 9%); adjuvant CHT and RT 73% versus 37% (TNBC versus HR+/- HER2—)</td>
<td>TNBC versus HR+/HER-2— versus HR+/- HER2+: LR—3% versus 0.3% versus 4%; DR—13% versus 3.5% versus 16%</td>
<td>Decreased OS, RFS and DFS in both uni- and multivariate analysis in the TNBC; later data show decreased RFS (89% versus 98% TNBC versus HR+/HER2—) also in T1N0 TNBC tumors despite of 70% adjuvant CHT.</td>
</tr>
</tbody>
</table>
study among 3193 patients, a significantly elevated risk of CM among patients with TNBC and HER-2-positive BC compared with other phenotypes has been reported (HR 4.5 and 4.9 for TNBC and HER-2+, respectively) [76]; the risk of CM was particularly pronounced among young patients with node-negative disease: the incidence of CM among patients <50 years of age and node positive was 20.0% for TNBC compared with 4.8% for HER-2 positive. Importantly, diagnosis of CM among patients with TNBC compared with non-TNBC was followed by a shorter median survival of 3–5 versus 7–12 months, respectively [75, 76, 78, 79]. CM as first site of distant relapse may occur among as many as 3.5%–14% of patients with TNBC [75, 77]. Central nervous system (CNS) relapse in patients with TNBC showed survival times as low as 2.9 and 5.8 months in patients with CNS relapse as the first site and later site, respectively [77]. Similarly, an OS of 3 and 4 months after diagnosis of cerebral metastasis was shown in the presence or absence of systemic therapy, respectively [80]. Recently, a nomogram to calculate the probability for developing cerebral metastasis, particularly for patients with TNBC has been indicated [81]; the clinical implications of which, however, remain unclear.

clinically relevant aspects of molecular biology of TNBC: mutation in BRCA and p53

BRCA1 plays a central role in repair of double-stranded DNA breaks; a lack of BRCA1 therefore results in genomic instability thereby predisposing to the development of malignant disease. TNBC/BLBC phenotype is particularly associated with BRCA1 mutations [82]. Similarly, about three-quarters of BRCA1-related BCs exhibit a BL phenotype by gene expression microarray [9] or immunohistochemistry [83], particularly among younger patients and patients with a family history of BC who very often also present with p53 mutations. Conversely, in an unselected cohort of 177 patients with TNBC as many as 11.3% were demonstrated to carry a BRCA1 mutation [84]. Among patients <40–50 years of age, the incidence of BRCA1 mutations is even higher (11%–29%) [84, 85].

In gene expression-based analyses, all BRCA1-associated cases were profiled into the BL subtype, together with sporadic TNBC [60]. Of note, frequent cytogenetic aberrations typically found among BRCA1 mutation carriers such as deletion of 5q are found at a similar frequency in sporadic TNBC [86, 87]. p53 is part of a cell-cycle checkpoint, exhibiting a molecular response to DNA damage resulting in apoptosis. In several studies, p53 is mutated in up to 82% of BLBCs by gene [9] as well as protein [23, 61] expression analysis. This is reflected by an increased genetic instability, specific cytogenetic changes, and higher loss of heterozygosity (LOH) frequency in BLBC/TNBC [23]. For instance, gain of 6p21–p25 as well as loss of 5q11 are common findings among BLBC [88], the latter carrying several DNA-repair and suppressor genes. Interestingly, the spectrum of p53 mutations among BRCA1-mutated TNBC is distinct from that occurring in sporadic TNBC [89]. The ataxia–telangiectasia-mutated kinase is aberrantly reduced similarly in both BRCA1-mutant (33%) and...
BRCA1-mutant (30%) hereditary BCs as well as in sporadic TNBC (20%) compared with only 10% among others [90]. Of note, cases of sporadic TNBC also share biological as well as histological features with BCs in BRCA1 mutation carriers such as central necrosis, lymphocytic infiltrate, genomic instability, and loss of LOH [91, 92]. Furthermore, nongenetic BRCA1 dysfunction may occur among sporadic BLBCs [93]. Conversely, a significant association of BRCA1-mutated cases with typical molecular features of TNBC, such as EGFR/CK 5/6 expression, ER/HER-2 negativity, and p53 mutations [82, 94] has been reported.

Finally, although the majority of BCs in BRCA1 mutation carriers carry a TN/BL phenotype, it is important to recognize that the majority of TNBC is in fact sporadic. Consequently, Collins et al. [94] demonstrated that basal markers do not sufficiently predict for BRCA1 mutations.

**Chemotherapy in BLBC/TNBC**

**Chemosensitivity and the TN paradox**

Several studies have shown that TNBC/BLBC is associated with an increased response rate to (neoadjuvant) chemotherapy. In *vitro* studies indicated distinct response patterns to 5-fluorouracil (5-FU) and anthracycline chemotherapy in luminal and basal cell lines [95]. Rouzier et al. [16] stratified 82 patients according to the microarray-based molecular classification developed by Perou et al. In both BL and HER-2 subtypes, the authors showed pCR rates of 45% to preoperative paclitaxel/FAC chemotherapy compared with only 6% in luminal subtypes [16]. Similarly, Carey et al. reported clinical and pathological response rates for neoadjuvant anthracycline–cyclophosphamide-based (AC) chemotherapy as being significantly higher in ER- and HER-2-negative patients compared with other subtypes. Despite this, BL and HER-2-positive/ER-negative subtypes experienced a significantly decreased disease-free survival (DFS) (*P* = 0.04) and OS (*P* = 0.02) compared with patients with ER-positive luminal subtypes [62].

In the largest study to date regarding this issue, Liedtke et al. examined the association between the TNBC and response to several regimens of neoadjuvant chemotherapy as well as OS in 1118 patients with early-stage BC. Again, although an increased pCR rate was observed for TNBC, patients in the TNBC subgroup showed decreased survival rates compared with non-TNBC. Interestingly, patients experiencing pCR following neoadjuvant chemotherapy had an excellent OS regardless of receptor expression; in contrast, patients who had residual invasive carcinoma after completion of neoadjuvant chemotherapy had a significantly shorter OS associated with TNBC compared with non-TNBC [47]. This clearly demonstrates that the poor OS of TNBC is derived from the fraction of patients with chemoresistant disease unfortunately representing >50% of TNBC.

This observation underscores two important issues. First, novel diagnostic tools need to be developed allowing for the identification of those patients that are not sensitive to existing chemotherapies and are in need of alternative treatment options. Secondly and consequently, these patients require the development of novel therapeutic tools.

**Chemosensitivity and tumor grade in TNBC**

Recently, a multigene index representing a genomic correlate of histological tumor grade has been established [96, 97]. High GGI is predictive of response to chemotherapy across all BCs but since most TNBCs have high GGI, its predictive value within this subset is limited. Also, a subgroup of TNBC shows resistance to taxane–anthracycline-containing chemotherapy despite high grade [98] indicating that some TNBCs carry additional molecular features overriding the increased chemosensitivity generally associated with high tumor grade. For instance, newer data support a presumable association between response to chemotherapy and the extent of the local immune reaction within the TN tumor indicating tumor-infiltrating lymphocytes and level of tumor cell apoptosis as predictive markers for response to neoadjuvant chemotherapy [99].

**TN and BL status as predictive markers for chemotherapy sensitivity**

The I-SPY trial is a multicenter trial designed specifically to identify predictive markers for both pCR and survival among women with locally advanced BC and is the first trial comparing the predictive value of TNBC and BL status [100]. Recent analyses by Esserman et al. demonstrated that patients with TNBC as well as BLBC can expect similarly favorable pCR rates of 33% and 34%, respectively, following anthracycline-taxane-based neoadjuvant chemotherapy which are significantly increased compared with those in HR-positive/luminal disease. Response to neoadjuvant chemotherapy measured either by dichotomization into pCR/residual disease (RD) or as a four-tiered response score [101] provided significant prognostic information among patients with BLBC; patients with excellent response to neoadjuvant chemotherapy had an excellent relapse-free survival (RFS); patients with extensive RD (RCB-III) died within 18 months (*P* < 0.0001) [100].

**Chemosensitivity to anthracyclines and cyclophosphamide**

Whereas patients with HER-2-overexpressing and/or topoisomerase-IIa-abnormal BCs have repeatedly been indicated to derive the most pronounced benefit from anthracycline-containing chemotherapy [102, 103]; results on the efficacy of anthracycline-based regimens in patients with TNBC remain controversial. A recent meta-analysis from four studies investigating anthracycline-containing regimens versus cyclophosphamide–methotrexate–5-fluorouracil (CMF) showed that although benefit from anthracyclines was pronounced among patients with HER-2-positive disease, patients with TNBC still experienced a substantial 23% reduction in the risk of disease relapse (*P* = 0.11) [104]. In the neoadjuvant setting, anthracycline-based regimens both with [16] and without taxanes [62] in this group are similarly efficacious. For instance, pCR rates after four to six courses of cyclophosphamide–epirubicin–5-fluorouracil (CEF) were 17% for patients with TNBC [105]. Of note, Berrada et al. studying 823 patients receiving six cycles of CEF or no chemotherapy identified p53+/BLBC as one subgroup deriving particular benefit from this chemotherapy [106].
Similarly, as enhanced response rates to anthracyclines may be achieved by increasing either dose intensity/density of the applied chemotherapy, an increase in pCR rate from 13% to 47% by intensifying conventional neoadjuvant \( \text{FE}_{100C} \) chemotherapy to \( \text{FE}_{270C} \) mg/m² (d1+8) in combination with standard 5-FU (d1-5) has been reported [107]. The WSG AM 01 trial randomly assigned patients with more than nine involved lymph nodes to receive either dose-dense conventional chemotherapy (i.e. 4× EC followed by 3× CMF q2w) or a rapidly cycled tandem high-dose regimen (i.e. 2× EC q2w followed by 2× Epirubicin+4× Cyclophosphamide+4× Thiotepa q3w). In this study, young patients with TNBC and/or G3 tumors derived greater benefit from the rapidly cycled tandem approach than from the dose-dense conventional regimen. The high-dose approach lead to 5-year event-free survival rates as high as 71% in patients with TNBC compared with only 26% in TNBC patients treated by conventional dose-dense chemotherapy [108].

Of note, a retrospective analysis from the MA5 trial randomly assigning patients to receive either CMF or CE adjuvant chemotherapy indicated an increased 5-year DFS for the former (71% versus 51%, respectively) among patients with BLBC; the test for interaction between BL phenotype and treatment arm reached borderline significance \( (P = 0.06) \) indicating that patients with TNBC may not derive a particular benefit from anthracyclines [109]. Although these retrospective results challenge the role of anthracyclines in adjuvant therapy for TNBC/BLBC, additional data will be needed for final clarification of this issue.

**chemosensitivity to taxanes**

To date, there are limited data from randomized clinical trials investigating the impact of implementing taxanes into the adjuvant setting in patients with TNBC. Hayes et al. [110] illustrated that patients with either TN or HER-2-positive BC derived the greatest benefit from the addition of four cycles of paclitaxel to four cycles of escalating doses of doxorubicin combined with a fixed dose of cyclophosphamide (AC) in 3170 node-positive patients. Similarly, Citron et al. showed that the same dose-dense schedule particularly benefited patients with ER-negative tumors at an overall relative reduction in the hazard of recurrence of 32% and 19% for ER-negative and ER-positive BCS, respectively. However, this difference by ER status did not reach statistical significance [111].

In a retrospective analysis regarding the PACS 01 trial comparing six cycles of \( \text{FE}_{100C} \) to three cycles of \( \text{FE}_{100C} \) followed by three cycles of docetaxel in node-negative BC, 33 markers were applied to stratify patients into two distinct molecular subgroups (‘basal’ and ‘luminal’). Despite a significantly overall decreased DFS, patients with BL compared with luminal tumors benefited more from the addition of docetaxel to standard FEC\( _{100C} \) chemotherapy \((HR = 0.65; P = 0.009)\) [65].

The BCIRG 001 trial compared six cycles of TAC versus CAF in node-positive BC; in this study, patients with TNBC experienced a 3-year DFS rate of 73.5% after six cycles of TAC compared with 60% after six cycles of FAC \((HR = 0.50, P = 0.051)\) [63]. These data are corroborated by an excellent pCR to neoadjuvant six or eight cycles of TAC (supplemented by capecitabine/vinorelbine in those patients not responding after two cycles of TAC) among patients with TNBC in the GEPARTRO trial (40.7% versus 31.6%), particularly in patients <40 years of age (60.0%) [112]. Similarly, results from the GEICAM 9906 trial show that eight cycles of weekly paclitaxel after four cycles of CEF versus six cycles of CEF are significantly more effective in patients with TNBC \((HR = 0.58, P = 0.025)\) [113]. These data on weekly paclitaxel administration are particularly interesting following presentation of the results of the trial comparing conventional 4× AC followed by 4× paclitaxel q3w versus 4× Apaclitaxel followed by 12× P weekly. A particular benefit of weekly paclitaxel was obtained for TNBC (5-year DFS 87% versus 79%, \( HR = 0.59, P = 0.037 \)) [114]. This is in line with recent data regarding weekly paclitaxel after four cycles of AC indicating that the benefit of paclitaxel q1w (but not docetaxel) compared with paclitaxel q3w was pronounced in the TNBC and HR+/HER-2 subgroups [115].

In patients with metastatic TNBC resistant to anthracycline-based or taxane-based chemotherapy, Rugo et al. [116] reported improved progression-free survival (PFS 4.1 versus 2.1 months) and overall response rate (ORR 27% versus 9%) for the novel microtubule-stabilizing agent ixabepilone in combination with capecitabine compared with capecitabine alone as current standard in this situation. Similarly, in the neoadjuvant setting, a 26% pCR rate was observed among patients with TNBC [117]. As a consequence of these data, the PACS 08 trial has been designed as a randomized phase III trial evaluating the benefit of a sequential CE\( _{100F} \) and ixabepilone chemotherapy compared with CE\( _{100F} \) followed by three cycles of docetaxel in the adjuvant treatment of patients with TNBC.

Loss or inactivation of \( \text{BRCA}1 \) function is thought to be associated with particular sensitivity to DNA-damaging (e.g. alkylating) chemotherapy [118]. Sensitivity of \( \text{BRCA}1 \)-mutated cells to microtubule agents, like taxanes or vinca alkaloids, however, remains controversial. In *in vitro* evidence on \( \text{BRCA}1 \) genotype-specific sensitivity to commonly used chemotherapy drugs stems from both human cell line and murine tumor models indicating that \( \text{BRCA}1 \) mutations may confer resistance against taxanes [119–121]. Despite this, to date, there is no convincing clinical evidence regarding a decreased sensitivity to taxanes [45] in TNBC versus non-TNBC.

A retrospective study investigated the effect of neoadjuvant EC chemotherapy in Ashkenazi Jews. An impressive pCR rate of 92% (10 of 11 patients) was reported in hereditary (\( \text{BRCA}1 \) or \( \text{BRCA}2 \) mutated) BC compared with only 30% in 38 sporadic controls [122]. Similarly, Rodriguez et al. showed that a \( \text{BRCA}1 \) gene expression signature (as a surrogate of \( \text{BRCA}1 \) mutation status) could subdivide sporadic TNBC into two groups; the majority of responses to preoperative EC was observed among patients carrying the \( \text{BRCA}1 \) signature (four of five pathological responders) [123]. In contrast, a recent study investigating the effect of neoadjuvant CE\( _{100F} \) in 393 patients (55 TNBCs, 14 of which had a \( \text{BRCA}1 \) mutation) reported a 44% pCR rate for patients with TNBC overall compared with only 17% for those with TNBC/\( \text{BRCA}1 \)-deficient tumors indicating that \( \text{BRCA}1 \) deleterious mutations may decrease
efficacy of anthracycline-based chemotherapy in patients with TNBC [124]. A very recent publication by Kriege et al. [125] shows similar response rates for 93 metastatic BRCA1 carriers for mostly used anthracycline-based and CMF chemotherapy compared with sporadic controls.

**platinum-containing agents**

The association of TNBC with BRCA1 mutations and dysfunctional DNA repair may indicate an increased sensitivity toward DNA-damaging agents, i.e. platinum agents. A recent preclinical study demonstrated that overexpression of p63 (a p53-related transcription factor) and p73 (p53 associated as well) is common among TN cases and associated with sensitivity to cisplatin [126].

Clinical data regarding the use of platinum agents in TNBC are still limited. A summary of these data is given in Table 3. In summary, despite an increasing amount of data indicating platinum agents as carrying particular efficacy in BLBC/TNBC, there are yet no randomized data identifying platinum-based chemotherapy as optimal regimen. Moreover, despite these encouraging results, safety concerns remain regarding these combined treatment modalities, although investigators overall report a manageable toxicity profile.

**resistance mechanisms**

Growth factors such as EGFR, c-kit, or p53 mutation status and several proliferative mechanisms like mitogen-activated protein kinase (MAPK) and protein kinase components of the extracellular signal-regulated kinases (ERK) pathway have been indicated as possible determinants of sensitivity to chemotherapy in TNBC [139, 140]. Furthermore, small heat-shock protein alpha B crystalline inducing epidermal growth factor-independent cell growth, migration and invasion and constitutively activating the MAPK/ERK pathway in vitro is commonly expressed in TNBC. Its expression is associated with resistance of tumors to preoperative chemotherapy (AC or AC-paclitaxel) in vivo [141]. A recently published study supports MAPK and phosphatidylinositol 3-kinase as potential targets for BLBC and also underscores the role of phosphatase and tensin homolog for response [142].

ABI-007 (nab-paclitaxel) is a novel nanoparticle albumin-bound (nab) formulation of paclitaxel [143]. Caveolin-1 is commonly found in cell membrane invaginations (caveolae) [144] providing an endocytic and exocytic compartment at the cell surface compartmentalizing a variety of signaling activities. Importantly, it may act as a mediator of transcytosis/extravasation of drugs like ABI-007. Given that caveolin-1 is frequently expressed by TNBC [145], ABI-007 may be of potential relevance regarding TNBC; however, this remains to be demonstrated in the context of clinical trials (www.clinicaltrials.goc).

**possible targeted therapies**

Given that patients with TNBC resistant to chemotherapy are in need of effective novel therapeutic agents to prevent them from particularly poor prognosis. Several biologically targeted agents are currently explored in this group.

**PARP inhibitors**

Single-strand DNA breaks are usually removed by base excision repair, of which poly-ADP-ribose-polymerase (PARP)-1 represents one of the central components. In the absence of PARP, single-strand breaks may degenerate to double-strand breaks, which cannot be repaired in BRCA1-mutated cells. Preclinical evidence indeed indicates BRCA1-null cells to be particularly sensitive to PARP1 inhibitors [146]. Furthermore, pretreatment with a PARP inhibitor can enhance the effect of cisplatin chemotherapy in vitro in preclinical models using BC cell lines [147]. Consequently, a number of clinical trials are currently being conducted with PARP inhibitors either alone or in combination with platinum-based chemotherapy, some of which have already provided promising results [148].

Results of two very important clinical trials implementing PARP inhibitors in patients with metastatic BC have recently been reported (for details see Table 4). These phase II results are promising but will need to be validated in larger possibly phase III trials.

**EGFR**

TNBC is strongly associated with EGFR expression. Yet, the most benefit of tandem high-dose chemotherapy was shown among TNBCs but not in the small subgroup of EGFR-positive tumors indicating the need for additional targeted therapies in this fraction [108]. In view of the small numbers and the methodological difficulties regarding EGFR testing (e.g. determination of activated versus total expression) [152], our results need to be substantiated before definite conclusions are possible. A body of preclinical data support a synergistic effect of EGFR inhibition (e.g. by use of the tyrosine kinase inhibitor gefitinib) with chemotherapy showing improved efficacy compared with chemotherapy or targeted therapy alone [153]. In the same study, both EGFR inhibitors cetuximab and erlotinib demonstrated very limited efficacy when employed as single agents. Given the increased efficacy of platinum-based drugs in BRCA1-deficient BC and preliminary preclinical results indicating enhanced radiosensitivity by use of cetuximab [154] in BLBC/TNBC, a number of clinical trials have been designed implementing EGFR-targeted agents into the treatment of TNBC. Details of these two trials are presented as part of Table 4. In summary, on the basis of the available evidence, there is little reason to believe that either single-agent cetuximab or a small-molecule tyrosine kinase inhibitor of EGFR will show substantial single-agent activity in patients with TNBC. Efforts to examine the effect of EGFR-targeted treatments on chemotherapy sensitivity are currently being conducted. Until the results of these clinical trials are presented, it remains unknown whether EGFR-targeted agents add any value to the therapy for TNBC.

**multityrosine kinase inhibitors**

The multitryosine kinase inhibitor dasatinib is a small molecule that has recently been approved for the treatment of bcr-abl-mutated chronic myeloid leukemia resistant to imatinib.
Table 3. Platinum-containing chemotherapy in TNBC

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Setting</th>
<th>Regimen</th>
<th>Efficacy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven BRCA1 mutation</td>
<td>N = 25 [127]</td>
<td>Neoadjuvant</td>
<td>4× cisplatin q3w</td>
<td>pCR rate: 72%</td>
<td>40% cT1 tumors, 70% cN0</td>
</tr>
<tr>
<td>Any TNBC</td>
<td>N = 28 [128]</td>
<td>Neoadjuvant</td>
<td>4× cisplatin q3w</td>
<td>pCR rate: 22%</td>
<td>Higher efficacy of cisplatin in patients with low BRCA1 mRNA and/or BRCA1 methylation [75]</td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBC with LABC</td>
<td>N = 30 [129]</td>
<td>Neoadjuvant</td>
<td>4× epirubicin/cisplatin/5-FU → 3× paclitaxel q1w</td>
<td>pCR rate: 40%; ORR: 86%</td>
<td></td>
</tr>
<tr>
<td>TNBC with LABC</td>
<td>N = 55 [130]</td>
<td>Neoadjuvant</td>
<td>4× cisplatin/bevacizumab q3w</td>
<td>Response ratea 36%</td>
<td>5-year DFS of 90% (pCR) versus 56% (non-pCR)</td>
</tr>
<tr>
<td>TNBC with LABC</td>
<td>N = 74 [131]</td>
<td>Neoadjuvant</td>
<td>8× cisplatin/ epirubicin/paclitaxel q1w+G-CSF</td>
<td>pCR rate: 62%</td>
<td></td>
</tr>
<tr>
<td>TNBC with LABC</td>
<td>N = 10 [132]</td>
<td>Neoadjuvant</td>
<td>4× Taxotere/carboplatin q3w</td>
<td>pCR rate: 50%</td>
<td>Mean tumor size 8.1 cm</td>
</tr>
<tr>
<td>TNBC with LABC</td>
<td>N = 125 [133]</td>
<td>Neoadjuvant</td>
<td>4× Taxotere/carboplatin or cisplatin with 4× AC q3w (A) (n = 76) or without (B) (n = 42)</td>
<td>pCR—A: 40%, no AC: 29%</td>
<td>Worse survival carboplatin versus cisplatin group</td>
</tr>
<tr>
<td>Any</td>
<td>N: N = 94 (n = 17 TNBC); M: N = 155 (n = 34 TNBC) [134]</td>
<td>Neoadjuvant (N) and metastatic (M)</td>
<td>N: 6× 5-FU/epirubicin/cisplatin q3w; M: mitomycin/vinblastin/ cisplatin or carboplatin</td>
<td>N: CR rate 88% versus 51% (TNBC versus non-TNBC); M: ORR 41% versus 31% (TNBC versus non-TNBC) (P = 0.3)</td>
<td>N: 60% of TNBC patients had no surgery, worse survival in TNBC; M: better 6-month PFS in TNBC</td>
</tr>
<tr>
<td>Any</td>
<td>N = 106 (n = 36 TNBC) [135]</td>
<td>Metastatic (first and second line)</td>
<td>Mostly paclitaxel/carboplatin or cisplatin</td>
<td>ORR: TNBC 37.5%, overall 39%</td>
<td>Median PFS—TNBC versus HR+ 6.2 versus 9.1 months OS; TNBC versus others: 21 versus 56 months</td>
</tr>
<tr>
<td>Any</td>
<td>N = 33 (n = 68 total) [136]</td>
<td>Metastatic (60% first line)</td>
<td>Irinotecan/carboplatin (part of study with cetuximab)</td>
<td>ORR: TNBC 30%, HR+ 31%</td>
<td>Median PFS—TNBC versus non-TNBC: 5.1 versus 4.1 months</td>
</tr>
<tr>
<td>TNBC</td>
<td>N = 44 [137]</td>
<td>Metastatic (~60% first line)</td>
<td>Gemcitabine/carboplatin (AUC2, d1/8) (part of study with PARP inhibitor)</td>
<td>ORR 16%</td>
<td>Median PFS 3.3 months, OS 5.7 months</td>
</tr>
</tbody>
</table>

TNBC, triple-negative breast cancer; pCR, pathological complete response; mRNA, messenger RNA; LABC, locally advanced breast cancer; 5-FU, 5-fluorouracil; ORR, overall response rate; G-CSF, granulocyte colony-stimulating factor; DFS, disease-free survival; N, neoadjuvant; M, metastatic; CR, clinical response; PFS, progression-free survival; OS, overall survival; PARP, poly-ADP-ribose-polymerase; AUC, area under the curve; q3w, every 3 weeks; q1w, weekly.

aResponse according to the Miller-Payne classification [138].
Table 4. Biological target agents in TNBC

<table>
<thead>
<tr>
<th>Patients</th>
<th>Setting</th>
<th>Regimen</th>
<th>Efficacy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP inhibitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven BRCA1 or BRCA2 mutation</td>
<td>N = 27; phase II [149]</td>
<td>Metastatic (median of three previous chemotherapies)</td>
<td>Olaparib 400 mg/m²</td>
<td>ORR 41%, median PFS 5.7 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNBC</td>
<td>N = 116; phase II [137]</td>
<td>Metastatic (~40% first line)</td>
<td>Gemcitabine/ carboplatin with (A) or without (B) BSI-201 (5.6 mg/kg d 1,4,8,11)</td>
<td>ORR A: 62% versus B: 21%; median PFS: 6.9 versus 3.3 months (HR = 0.342)</td>
</tr>
<tr>
<td>VEGF inhibitors</td>
<td>Any</td>
<td>Metastatic; all first line</td>
<td>Paclitaxel d 1,8,15 +/(A)/−(B) bevacizumab 10 mg/m²</td>
<td>Median PFS: 8.8 (A) versus 4.6 (B) months (HR = 0.47)</td>
</tr>
<tr>
<td>EGFR inhibitors</td>
<td>TNBC</td>
<td>Metastatic (46% first line)</td>
<td>Cetuximab 400 mg/m² → 250 mg/m²/mono → +carboplatin AUC2 q1w on progression (A) or up-front (B)</td>
<td>RR cetuximab mono: 6% versus combination (A or B): 17%</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>Metastatic (64% first line)</td>
<td>Irinotecan 100 mg/m²/ carboplatin AUC2.5 +/(A)/−(B) cetuximab 400 mg/m² → 250 mg/m²/mono</td>
<td>RR TNBC A: 49% versus B: 30%; median PFS A: 5.1 versus B: 4.7 months</td>
</tr>
</tbody>
</table>

TNBC, triple-negative breast cancer; PARP, poly-ADP-ribose-polymerase; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; VEGF, vascular endothelial growth factor; ER, estrogen receptor; PgR, progesterone receptor; RR, response rate; EGFR, epidermal growth factor receptor; SD, stable disease; +(A)/−(B), with Arm A and without Arm B; Mono, monotherapy.

In vitro evidence supports the use of this small molecule particularly in subgroup of BLBC in which several tyrosine kinase receptors such as stem-cell factor receptor (c-kit) are overexpressed and/or mutated [155, 156]. A single phase II study evaluating single-agent dasatinib in patients with advanced TNBC reported modest activity, with a partial remission among two and a clinical benefit among six patients (overall clinical benefit rate 9.3%); however, a discontinuation of therapy and dose reductions weaken the results of the study [157]. Impressive preclinical data on response of TNBC xenografts to the heat-shock protein 90 inhibitor PU-H71 by inhibition of the Ras/Raf/MAPK pathway and G(2)–M phase and reduction of the invasive potential thus leading to complete response and tumor regression has also been reported [158].

antiangiogenic agents
Antiangiogenic therapy provides a further candidate mechanism for improving treatment efficacy in patients with TNBC. For instance, Linderholm et al. [159] reported increased levels of vascular endothelial growth factor (VEGF) in patients with TNBCs which were associated with shorter RFS in patients with TNBC compared with those with non-TNBC, indicating a positive correlation of expression of angiogenic factors (i.e. CD31 and CD105) in high-risk BLBC (O. Gluz, A. Gaumann, A. Hartmann, et al., unpublished data). Recently, a particular association between VEGF signaling and chromosome organization related to gene gains in 6p21–22 in the TNBC phenotype was shown which may represent potential pathway targets in this subtype [161]. The monoclonal anti-VEGF antibody bevacizumab in combination with weekly paclitaxel caused a significant increase in response rates and PFS in all subgroups with metastatic BC [150]. The study of Avastin (Bevacizumab) Adjuvant Therapy in Triple Negative Breast Cancer (BEATRICE) was designed to investigate the effect of adding bevacizumab to adjuvant chemotherapy in breast cancer (ABC0-04) [121].

Also, VEGF-2 has been reported by the same group as a prognostic factor among patients with TNBC indicating vascular pathways as one very interesting mechanism for targeting this BC subtype [160]. Similarly, our own data indicate a positive correlation of expression of angiogenic factors (i.e. CD31 and CD105) in high-risk BLBC (O. Gluz, A. Gaumann, A. Hartmann, et al., unpublished data). Recently, a particular association between VEGF signaling and chromosome organization related to gene gains in 6p21–22 in the TNBC phenotype was shown which may represent potential pathway targets in this subtype [161]. The monoclonal anti-VEGF antibody bevacizumab in combination with weekly paclitaxel caused a significant increase in response rates and PFS in all subgroups with metastatic BC [150]. The study of Avastin (Bevacizumab) Adjuvant Therapy in Triple Negative Breast Cancer (BEATRICE) was designed to investigate the effect of adding bevacizumab to adjuvant chemotherapy in TNBC.

Finally, sunitinib malate is an oral multitargeted tyrosine kinase inhibitor inhibiting vascular endothelial growth factor receptor, platelet-derived growth factor receptor, c-kit, and colony-stimulating factor-1 receptor. In a recently published phase II study, moderate activity of monotherapy (ORR of
TN/BL disease is a BC subtype with a significantly increased risk of relapse or disease progression as well as distinct patterns of metastatic progress, leading to early visceral and CNS involvement. However, clinical and molecular heterogeneity exists within this subtype. A substantial minority of these cancers is highly sensitive to existing chemotherapies and their survival can be excellent if treated adequately as evidenced by the good long-term survival of patients with TNBC who achieve pCR to preoperative chemotherapy. The optimal chemotherapy regimen for these cancers remains to be determined. For the time being, a standard third-generation taxane-including adjuvant chemotherapy may be the most appropriate. The role of platinum agents as adjuvant treatment is currently being defined. To date, chemotherapy and bevacizumab if available are the only currently approved therapy options for metastatic TNBC. Optimal schedule and regimens remain unclear. At present, there are no randomized data justifying omission of anthracyclines or replacement thereof by alternative agents, such as platinum agents, outside of clinical trials, particularly in the potentially curable adjuvant setting. BRCA1 and several proliferation mechanisms play a crucial role in therapy response of TNBC/BLBC and are discussed to mediate sensitivity to DNA-damaging agents (platinum, alkylating agents). Furthermore, novel targeted therapies (e.g. PARP, EGFR, c-kit and VEGF inhibitors alone or in combination with chemotherapy) are currently under investigation and have shown promising results in numerous phase II trials.

... and future directions:

It seems to be plausible that the poor outcome observable among patients with TNBC can be partly attributed to BL tumors within this clinically defined subgroup; however, to date, there is no consensus regarding the methodology for defining BLBC and clinical consequences for this subtype remain unclear. There is an urgent need to identify distinct molecular features to stratify distinct BC subtypes with patients with TN disease in order to improve identification, subgrouping, and treatment of patients with TNBC. Furthermore, scientific efforts in the near future will have to focus on the incorporation of better imaging techniques (e.g. very controversial use of MRI for detection of high-grade DCIS as a precursor of TNBC [163]), especially in high-risk collectives. Furthermore, given the substantial risk of cerebral metastasis and the associated poor prognosis, innovative clinical trial concepts such as prophylactic cranial irradiation (at least in patients with chemotherapy-resistant tumors) may be justified despite the controversy [78]. Furthermore, blood–brain barrier-crossing substances such as thiotepa may be incorporated into chemotherapy regimens for TNBC specifically.

We have shown that patients who do not achieve complete pathological remission have a rather poor outcome requiring alternative/additional (chemo-)therapy options rather than standard treatment. Consequently, two questions need to be addressed:

- Who are the patients that do not benefit sufficiently from standard chemotherapy?
- What alternative therapies can we offer them?

To address the first question, improved strategies required for early recognition of responders and nonresponders such as novel imagining techniques like PET [164] and/or MRI may be reevaluated in the context of TN disease. To address the second, several concepts are possible:

- First, there is evidence that patients with TNBC derive particular benefit from dose-dense (weekly or biweekly) and other intensified chemotherapy regimens with standard agents, although the best strategy to select patients for these more toxic and cost-intensive therapies remains to be defined.
- Secondly, BRCA1 and several proliferation mechanisms play a crucial role in therapy response of TNBC/BLBC and are discussed to mediate sensitivity to DNA-damaging agents (platinum, alkylating agents).
- Thirdly, novel targeted therapies (e.g. PARP, EGFR, c-kit and VEGF inhibitors alone or in combination with chemotherapy) are currently under investigation and have shown promising results in numerous phase II trials.
- Last but not least, it remains to postulate that the optimal therapeutic concept for TNBC will eventually consist in a combination approach of all the above.

16%) in metastatic disease resistant to anthracyclines and taxanes was shown with a particular impact in patients with TN and HER-2-positive tumors [162].

acknowledgements

The authors state no potential conflict of interest regarding the contents presented in this review.

references

cyclophosphamide followed by paclitaxel or paclitaxel followed by weekly paclitaxel as adjuvant therapy for Annals of Oncology Volume 20


