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Therapeutic Advances and New Directions for Triple-Negative Breast Cancer

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Summary

Triple-negative breast cancer (TNBC) is a molecularly diverse grouping with poor prognosis for which chemotherapy remains the foundation of treatment. The molecular heterogeneity of the disease rationalizes its diverse biological behavior and differential response to treatment. Estimates of up to 20% of patients diagnosed have germline mutations in DNA-damage repair-pathway genes, namely *BRCA1* and *2,* and this can be used to select patients likely to respond to platinums and/or inhibitors of poly(ADP-ribose) polymerase (PARP). Similar strategies can be utilized in other subtypes of TNBC that have 'BRCA-like' tumor biology due to the presence of mutations in alternate DNA-damage repair genes. The diverse biological behavior of TNBC and its variable response to chemotherapy were largely decoded following genotyping studies that enabled the identification of distinct molecular subtypes, such that the biological and genetic heterogeneity of the disease could be understood. This subsequently enabled the identification of therapeutic 'vulnerabilities' for each subtype that encompass biological processes including proliferation, DNA repair, apoptosis, angiogenesis, immune modulation, and invasion and metastasis. To expedite the development of therapies for high-risk, early-stage breast cancer, we have adopted novel trial designs and re-defined endpoints as surrogates of clinical outcomes. The purpose of this review is to highlight the current standard and experimental treatment options for TNBC.

Introduction

Triple-negative breast cancer (TNBC) is defined as lacking the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu) protein or *HER2/neu* gene amplification. It comprises approximately 15–20% of all breast cancers in the United States [1]. The most strict definition of TNBC is provided by the American Society of Clinical Oncology and American College of Pathology (ASCO-CAP) as ER/PR-negative disease by immunohistochemical (IHC) analysis, specifically at < 1% expression of tumor cell nuclei immunoreactive for ER or PR [2] and HER2 1+ or 2+ by IHC but subsequently negative by *in situ* hybridization methods. The clinical and molecular characteristics of the disease are summarized in supplementary table 1 *(www.karger.com/?DOI=455821).* Younger women and those of black race or Hispanic ethnicity are more commonly affected [3–5]. TNBC is sensitive to chemotherapy; however, it is associated with a higher risk of distant recurrence, high rates of visceral and central nervous metastases, earlier time to recurrence, and worse prognosis after recurrence, indicating an aggressive clinical course compared to hormone receptor-positive subtypes [6–8]. This is often referred to as the triple negative paradox. Over 80% of breast cancers among patients with a hereditary *BRCA1* mutation are TNBCs [9]. Even sporadic TNBC shares many clinical and molecular features with BRCA1-associated cancers, including defective DNA repair, which may be due to methylation-induced silencing of *BRCA* or mutations in other DNA-repair genes [10, 11].

Aside from *BRCA1/2* mutation status, biomarkers to identify patients most likely to respond to current chemotherapy have not been identified and to date no FDA-approved targeted therapies are available for TNBC.

However, recent research has deciphered the molecular heterogeneity of the disease and these findings provide insight into its diverse biological behavior and differential response to chemother-

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apy [12, 13]. Novel targets are being elucidated, some of which have great potential for therapeutic development. Current clinical practice is moving toward implementation of molecular testing at diagnosis to define a personalized tumor-specific genetic 'fingerprint' that has the potential to identify molecular dependencies amenable to therapeutic intervention. Thus, the translational cancer research community is increasingly adopting a combined systems biology and integrated analysis approach to understand and predictively model the activity of cancer cells (supplementary fig. 1; *www.karger.com/?DOI=455821).* Advancing therapeutic strategies in the treatment of breast cancer with aggressive biology involves a commitment to defining and re-defining the molecular signature of disease at multiple points along its evolutionary lineage, so that therapy can be tailored to a changing tumor microenvironment. Novel trial design and re-defined endpoints as surrogates of clinical outcome have been introduced to expedite the development of breakthrough therapies to treat high-risk, early-stage breast cancer.

For this review, PubMed, MEDLINE and EMBACE were searched. Abstracts published in the proceedings of annual meetings of the American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), and the San Antonio Breast Cancer Symposium (SABCS) were reviewed. We also considered all relevant ongoing clinical trials registered in the ClinicalTrials.gov.

Molecular Classification of Breast Cancer

Several groups have made substantial progress in unraveling the diversity of TNBC and relating gene expression patterns to molecular or genotypic subtype [12–15]. Initial molecular classifications of breast cancer using PAM50 gene expression assigned most TNBCs into the basal-like (BL) group, with the remainder divided between the luminal and HER2-enriched groups [16]. Approximately a third of BL tumors exhibit loss of function of BRCA 1 or BRCA 2, both key regulators of homologous recombination, and these tumors demonstrate high sensitivity to both alkylating agents that induce DNA double-strand breaks (DSBs) and inhibition of poly(ADP-ribose)polymerase (PARP) [17, 18]. They are not sensitive to microtubule inhibitors such as taxanes [19]. A fifth subtype, denoted claudin-low, identified a cohort of tumors enriched for expression of mesenchymal genes [20, 21].

An alternate analysis of 21 breast cancer data sets containing 587 TNBC cases led to an enhanced molecular refinement encompassing 7 subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (M), mesenchymal-stem cell-like (MSL), immunomodulary (IM), luminal androgen receptor/luminal-like (LAR) and unclassified [12]. Functional analysis of genomic signatures, coupled with annotation of mutational and gene rearrangements for each subtype, facilitated cell and mouse tumor model-based evaluation of potential 'actionable targets' using a range of therapeutic agents (supplementary table 2; *www.karger.com/?DOI=455821).* Furthermore, a genomic based predictor, TNBCtype [22], was launched aimed at enabling oncologists to decipher TNBC molecular subtype that could guide treatment choice. These studies have reinforced the interest in platinum agents for the treatment of TNBC [23–25].

More recently, this classification has been refined further into 4 subtypes (BL1, BL2, M and LAR) based on the finding that differences in genomic signatures for M, MSL and IM categories was caused by infiltrating lymphocytes [13] (supplementary table 3; *www.karger.com/?DOI=455821).* Furthermore, this has been accompanied by simplification of a predictor algorithm to a 101-component gene profile that has been used to independently investigate pathologic response rates in different subtypes [26]. Compared to all other subtypes, BL1 had a significantly greater rate of pCR while the BL2 subtype had a significantly lower rate of pCR using the revised TNBCtype4 gene annotation, emphasizing the urgent need for novel therapeutic interventions in the latter.

Next generation sequencing combined with genomic cataloguing initiatives, such the Cancer Genome Atlas (TCGA) and cBioPortal [27, 28], have enabled robust classification of the spectra of mutations in TNBC. Germline mutations in DNA-damage repair genes increase risk of breast and ovarian cancer, and recent analysis of 1,824 TNBC tumors unselected for family history revealed mutations in 14.6% of all patients. In addition to the well-documented BRCA1 and BRCA2 involvement, deleterious mutations were detected in 15 other genes that participate in homologous recombination, including *PALB2* and *BARD1, RAD51D, RAD51C,* and *BRIP1.* Patients with these germline mutations had earlier onset of disease and higher grade tumors than women without mutations [29]. Somatic mutations previously implicated in breast cancer have also been confirmed by large-scale *studies (PIK3CA, PTEN, AKT1, TP53, GATA3, CDH1, RB1, MLL2, MAP3K1,* and *CDKN1B),* while novel genes have been identified *(TXB1, RUNX1, CBFB, AFF2, PIK3R1, PTPN22, PTPRD, NF1, SF3B1,* and *CCND3) [*28, 30]. Most TNBC-associated somatic mutations are in *TP53, TTN,* and *PIK3CA* genes (55%, 14%, and 9%, respectively) [31], and rates are similar between responders and non-responders. Of note, *PIK3CA* mutations prevail in the patients with intact BRCA1 function.

Numerous studies have approached mapping the genomic landscape of TNBC such that it is estimated that approximately 20% of these malignancies have potentially 'clinically actionable' somatic lesions [30]. Their distribution varies in a continuous distribution and is unrelated to copy number abnormality, or tumor cellularity. Low-abundance alterations such as translocations have also been documented, e.g.*MAGI3-AKT3* fusion causing constitutive AKT activation that can be therapeutically targeted with an AKT small-molecule inhibitor [30]. Another area of interest and growth is documenting treatment-induced alterations in residual disease following standard neoadjuvant chemotherapy (NAC) treatment [32] to inform potential therapeutic approaches for dealing with resistant cells. The most frequent changes noted were: novel mutations in *ATM, KDM6A, AR, DPYD* and *TP53* genes; a *CDH1* splice site; increased copy number of *AKT* and *CCND* family members and co-amplification of *MYC* and *MCL1.* A recent study has identified

a 3-gene signature *(CCL5, DDIT4* and *POLR1C)* derived from residual tumor following NAC that is prognostic of distant recurrence-free survival [33]. In summary, genomic studies have enabled precise annotation of the disease that can radically alter the future of treatment. Ongoing initiatives to match an extensive portfolio of targeted agents with eligible patients based on 'tumor fingerprinting' are underway.

Cytotoxic Therapy

At present, cytotoxic chemotherapy remains the mainstay of treatment for operable and advanced TNBC. A number of agents have activity in localized and advanced disease, including anti-tubulins, anthracyclines, alkylating agents, antimetabolites, and platinums [34, 35]. Standard adjuvant and neoadjuvant regimens typically include an anthracycline (doxorubicin or epirubicin) plus an alkylating agent (cyclophosphamide), given either concurrently with a taxane (docetaxel) or sequentially before or after a taxane (docetaxel or paclitaxel). These result in the highest pCR rates when used in the neoadjuvant setting and lowest recurrence rates when used in the adjuvant setting [36–40]. Recent clinical trials signal that there may be gains yet to be made through addition of other chemotherapy agents to this repertoire.

Uniquely in TNBC, for which there are currently no targeted options for additional adjuvant treatment beyond chemotherapy, the use of the neoadjuvant platform may hasten new developments.

Anti-Tubulin Therapy

The role of anti-tubulin agents in TNBC has been studied retrospectively and in prespecified subgroup analysis from clinical trials. A retrospective analysis including 399 patients in 2 phase III studies comparing ixabepilone plus capecitabine with capecitabine alone showed an improvement in progression-free survival (PFS; median 4.1 vs. 1.7 months, hazard ratio (HR) 0.63, $p < 0.001$) and response rate (28% vs. 14%), but not improvement in overall survival (OS) in the 443 patients with measurable and non-measurable disease (median 10.3 vs. 9.0 months, HR 0.87, $p = 0.18$) [41] for the addition of ixabepilone.

The 301 study found no improvement in OS of eribulin compared to capecitabine in 1,102 patients with metastatic breast cancer (MBC) who had received (neo/adjuvant) anthracycline and had up to 2 lines of chemotherapy in the metastatic setting (median OS 15.9 vs. 14.5 months, HR 0.88, $p = 0.056$), although 284 patients with TNBC exhibited improved OS with eribulin in a prespecified subgroup analysis (median 14.4 vs. 9.4 months; HR 0.70, 95% confidence interval (CI) 0.56–0.91) [42].

Platinum Salts

Based on enhanced susceptibility of some subclasses of triple-negative and BRCA1/2 mutant tumors to DNA-damaging chemotherapy agents [12], recent studies have focused on the role of platinums as a component of NAC. Higher pCR rates were consistently observed in TNBC compared with non-TNBC patients, and the pCR rates of invasive carcinoma in the breast and axillary nodes have been shown to be associated with improved long-term outcomes for TNBC [43]. In addition, higher pCR rates are observed in BRCA mutation carriers compared with non-mutation carriers treated with such regimens [44, 45].

Several trials have evaluated platinum therapy including specifically BRCA mutation carriers and patients not selected by mutation status (supplementary table 4; *www.karger.com/?DOI=455821).* A small proof-of-concept neoadjuvant study of 25 BRCA1 mutation carriers showed a pCR rate of 72% with single-agent neoadjuvant cisplatin [46], which compared favorably to a 21% pCR rate in 28 unselected patients with TNBC, 2 of whom had BRCA mutations [24].

Telli et al. [25] reported a pCR rate of 36% in 80 patients with TNBC treated with the carboplatin-gemcitabine combination, including 47% in patients with germline BRCA mutations. In addition, 2 randomized phase II trials demonstrated increases in pCR rates with the addition of carboplatin to taxane-containing therapy in patients with sporadic breast cancer not selected by mutation status. The GeparSixto trial demonstrated that pCR increased by approximately 20% (59% vs. 38%; $p < 0.05$) when carboplatin was added to neoadjuvant taxane/anthracycline plus bevacizumab in 293 patients with TNBC. However, about half of patients receiving weekly carboplatin discontinued treatment because of significant toxicity, and breast conservation rates were not significantly impacted [47].

In the Cancer and Leukemia Group B (CALGB) 40603 randomized phase II trial, 493 patients with stage II/III sporadic TNBC were randomly assigned to standard weekly paclitaxel for 12 courses with or without the addition of carboplatin (AUC 6 every 3 weeks for 4 cycles), bevacizumab or the combination. All patients also received dose-dense doxorubicin-cyclophosphamide for 4 courses after paclitaxel and before surgery. Of note, the concomitant use was associated with markedly higher toxicity with significantly fewer patients receiving 11–12 doses of paclitaxel (P) when carboplatin was added, compared to the control group (< 65% in the PCarbo group -> Anthracycline (AC) vs. 85% in P->AC) The addition of carboplatin significantly increased the pCR rate (54% vs. 41%, $p = 0.0029$); similar benefits were observed in the absence (49% vs. 39%) and presence (60% vs. 43%) of bevacizumab, and no interaction was observed ($p = 0.52$), indicating a lack of a synergistic effect [23]. Although numerically more patients were deemed potential candidates for breast-conserving surgery when carboplatin was used (57% vs. 44%), the difference was not statistically significant, and the actual rates were not reported.

Platinum-containing agents are not regarded as a standard for neoadjuvant therapy of TNBC, at least at present, for several reasons. First, given that the addition of platinum results in added toxicity, the clinical benefits should be clear. There were conflicting data from long-term outcomes presented at a recent SABCS. An improved disease-free survival (DFS) with the addition of carboplatin (HR 0.56, 95% CI 0.33–0.96, median follow-up 35 months) was reported in GeparSixto, while there was no demonstrated im-

provement in event-free survival (EFS) with the addition of carboplatin (HR 0.84, 95% CI 0.58–1.22, median follow-up 39 months) in the CALGB/Alliance 40603 study [48, 49]. Both studies were underpowered for long-term outcome endpoints and, thus, it is challenging to interpret the data conclusively. Second, it is possible that the improvements in pCR rates may be a result of down staging of low-volume residual disease, which would not be predicted to translate into fewer recurrences. Third, pCR may not be associated with improved outcomes in BRCA1/2 mutation carriers, suggesting the inconsistency of its prognostic effect.

The TNT phase III trial randomized 376 patients with metastatic TNBC to docetaxel or carboplatin. In unselected TNBC patients, objective response rates (ORRs) were similar (35%) between the 2 agents; however, in the BRCA mutation carriers (n = 29) response rates to carboplatin were 68% compared to 30% for docetaxel.

It remains unclear how platinum should be incorporated and whether concomitant use of platinum could be used to substitute for anthracycline, taxane or an alkylator. The efficacy of platinum chemotherapy alone, relative to standard chemotherapeutic options, in germline mutant versus non-mutant BRCA1/2 TNBC is the subject of ongoing trials through the Translational Breast Cancer Research Consortium (TBCRC) trial 030 (NCT01982448) and TBCRC 031 (NCT01670500).

Sacituzumab Govitecan

Sacituzumab govitecan (IMMU-132) is an anti-Trop-2-SN-38 antibody-drug conjugate (ADC) that received FDA breakthrough therapy designation in February 2016 for the treatment of patients with TNBC following at least 2 treatments for metastatic disease. A phase II study continues to provide a promising median survival benefit in 60 assessable patients with metastatic TNBC who had received a median of 5 (range 2–12) prior lines of therapy. As of May 2016, the ORR for this group of patients was 33% (confirmed ORR was 28%), which is nearly double that reported for standard-of-care in this late-stage setting, and the median duration of response was almost 11 months. Median PFS was 5.6 months, which is almost twice as long as that for conventional therapy, based on historical data, and median OS was 14.3 months. The major toxicity was neutropenia, which was manageable and did not result in cessation of therapy [50]. A confirmatory phase III trial is planned.

Drug Resistance and Response to Therapy as a Pharmacodynamic Biomarker

Tumor heterogeneity is the major factor that contributes to both intrinsic and acquired resistance, and is a major barrier to curative therapy. Although sensitive populations of tumor cells may be eradicated, there is undoubtedly selective enrichment of residual tumor cells that are often genetically and histologically distinct from sensitive cells [21, 32, 51]. There is also evidence that some cytotoxic agents may promote epithelial-to-mesenchymal transition (EMT) and/or enrich for tumor, initiating cells that can promote metastasis [21], although it is likely that these cells existed in the initial population prior to therapy. Other agents are purported to reverse EMT, thereby suppressing metastasis [52, 53], albeit in cell and mouse tumor model-based contexts.

Resistance to kinase inhibitors is often mediated by feedback loops that are hard-wired to adapt to changes in activity within a signaling network [54], and such observations have been used to rationalize combinatorial strategies to circumvent these adaptations.

pCR was recognized by the FDA as an acceptable surrogate endpoint that supported accelerated approval, but required improved EFS as a condition for full approval [55]. This was supported by a recent meta-analysis of 12 trials that included 11,955 patients. Cortazar et al. [43] found a strong correlation between pCR and EFS and OS in both HER2/neu-positive disease and TNBC, although there was little association in the trial-level data analysis between increases in frequency of pCR and EFS (coefficient of determination (R^2) 0.03, 95% CI 0.00–0.25) and OS (R^2 0.24, 95% CI 0.00–0.70). In addition, the I-SPY2 program uses pCR as an endpoint to identify promising agents in phase II trials that may be 'graduated' to more definitive evaluation in phase III trials.

Although achieving a pCR after NAC is associated with a favorable prognosis, the prognosis for patients with residual cancer is variable, and differs by molecular subtypes [56, 57]. The 5-year recurrence rate is significantly higher for patients with extensive residual disease compared with patients with no, or minimal, residual disease after NAC, especially in ER-negative disease, as shown in supplementary fig. 2 *(www.karger.com/?DOI=455821)* [58]. The risk of distant recurrence can be as high as 40–50% with the first 3–5 years. There is no proven role for continuing systemic therapy for patients with extensive residual TNBC who remain at high risk for recurrence despite receiving a course of taxane and anthracycline-containing NAC.

In addition, the powerful prognostic effect of pCR has led to it being used to select patients for clinical trials. An example of this approach is the CREATE-X study, a phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathological residual invasive disease after neoadjuvant anthracycline and/or taxane chemotherapy [59]. In that setting, capecitabine reduced the risk of recurrence and improved OS, including in patients with TNBC. If the results are confirmed, it could lead to a new therapeutic standard for high-risk TNBC.

There may be opportunities to evaluate the characteristics of residual disease to tailor specific therapies for patients who remain at high risk. For example, Balko et al. [32, 51] identified diverse molecular lesions and pathway activation in drug-resistant tumor cells and tumor from residual disease, providing a foundation for further evaluation of this strategy, as exemplified by a recent report evaluating cisplatin alone or in combination with the PARP inhibitor rucaparib [60]. Patients with residual TNBC despite standard alkylator plus anthracycline plus taxane chemotherapy are ideal candidates for clinical trials. Ongoing and planned studies in North America, such as the Eastern Cooperative Oncology Group-American College of Radiology Imaging Network (ECOG-ACRIN) EA1131 (NCT02445391) and SWOG (Southwest Oncology Group)

S1418, seek to define the role of capecitabine, platinum chemotherapy, or immune checkpoint inhibitors in patients with residual cancer after NAC. E1131 focuses only on patients that are truly at high risk for disease recurrence, hence sparing toxicity in the patients expected to have an optimal outcome with the current standard therapy. At the same time, patients with pCR to NAC represent an opportunity to study de-intensification of therapy, including reduction of the extent of locoregional treatment and the extent of obligatory adjuvant chemotherapy treatment. In the same context, the SWOG recently launched a randomized phase III trial to evaluate the efficacy and safety of MK-3475 as adjuvant therapy for TNBC with ≥ 1-cm residual invasive cancer or positive lymph nodes (>pN1MIC) after NAC.

Targeted Strategies

A variety of targeted therapies have been previously tested and are currently in development for TNBC, as summarized in supplementary table 4 *(www.karger.com/?DOI=455821)*. Successful implementation of this strategy for some agents may require the identification of predictive gene expression profiles, specific driver mutations, or other assays (supplementary table 5; *www.karger.com/?DOI=455821)* [13, 33].

Epidermal Growth Factor Receptor Inhibitors

Based on preclinical studies, the majority of TNBC tumors overexpress epidermal growth factor receptor (EGFR) [4, 61] and depend on it for proliferation. Despite that, clinical trials using anti-EGFR agents such as cetuximab demonstrated limited benefit [62–65]. A few studies have evaluated the efficacy of small-molecule EGFR inhibitors. Erlotinib had minimal activity in previously treated women with MBC [66], whereas gefitinib had modest activity [67].

Antiangiogenic Agents

The role of bevacizumab in the absence of a predictive biomarker is unclear. In 2008, the US FDA approved the use of bevacizumab in MBC; however, the decision was reversed based on the lack of supportive data beyond improvement in PFS. Studies such as the MeRiDian study to evaluate whether plasma vascular endothelial growth factor (VEGF) A could predict response were unsuccessful. In addition, although small-molecule VEGF inhibitors appear to be active in pretreated TNBC [68], in a phase III study the addition of sunitinib to capecitabine did not improve the clinical outcome of patients with MBC pretreated with anthracyclines and taxanes [69].

The addition of bevacizumab to NAC significantly increased the rate of pCR among patients with early-stage HER2-negative breast cancer, and most notably with TNBC [56], although these findings were not confirmed in the NSABP-40 [70], possibly due to different inclusion criteria and study design. Lastly, the primary results of a large international randomized phase III trial (BEATRICE) do not support adjuvant B in patients with TNBC [71].

PARP Inhibitors

The nuclear enzyme PARP is essential for the recognition and repair of DNA damage [72]; therefore, inhibition of PARP is hypothesized to potentiate the cytotoxicity of DNA-damaging agents. PARP nuclear enzymes are activated by DNA single-strand breaks or DSBs, resulting in the poly(ADP-ribosyl)ation of other nuclear DNA binding proteins involved in efficient DNA repair and survival [72–75]. 'Synthetic lethality,' the shutdown of the predominant DNA repair pathways that confer augmented cell death/apoptosis, may explain why BRCA1 or BRCA2 mutant cells are extremely sensitive to PARP1 inhibition [74]. Preclinical and clinical data support this hypothesis and the promising emerging potential of PARP as a therapeutic target for metastatic TNBC. At least 5 PARP targeting drugs are currently in clinical development (supplementary table 5; *www.karger.com/?DOI=455821)*.

Iniparib was purported to be a PARP inhibitor that initially showed promising results in randomized phase II trials in patients with TNBC [63], such as in combination with gemcitabine/carboplatin. The phase III clinical trial that failed to meet the primary endpoint [64] reduced the initial optimism. Subsequently, cell-based experiments revealed that iniparib is not only structurally distinct from other PARP inhibitors and a poor inhibitor of PARP activity, but also exerts its cytotoxic effects via alterations in the metabolism of reactive oxygen species in cancer cells [76, 77]. Thus, the concept of targeting PARP to induce 'synthetic lethality' is still under exploration/clinical development, albeit with a drug that is a potent in vivo inhibitor of PARP.

Another oral PARP inhibitor, olaparib was shown to be efficacious and safe in early phase clinical trials. In a phase I trial, olaparib exhibited PARP inhibition and antitumor activity in cancer associated with the BRCA1/2 mutations and was well tolerated [17]. A multicenter phase II sequential cohort study of 54 patients with impaired BRCA1/2 provides positive proof of concept. The ORR was 41%, while the median PFS was 5.7 months on the optimal dose [78]. Objective responses were not noted in patients with sporadic advanced TNBC in a Canadian phase II non-randomized study [79]. Phase III trials testing olaparib in the adjuvant and metastatic setting in patients with germline BRCA1/2 mutations are currently under way (supplementary table 5; *www.karger.com/?DOI=455821)*.

Veliparib (ABT-888) is a small-molecule inhibitor of PARP1 and 2 and has been studied in early phase clinical trials that included patients with MBC. In an exploratory investigational new drug (eIND) study conducted by the National Cancer Institute (NCI) phase 0 program, a single dose demonstrated good oral bioavailability and was well tolerated. Statistically significant inhibition of PARP was observed in tumor biopsies and peripheral blood mononuclear cells at 25-mg and 50-mg doses. The design of phase I trials of veliparib as monotherapy as well as in combination with chemotherapy was guided by the pivotal biochemical and pharmacokinetic data generated by this novel approach. The rapid completion of this trial not only accelerated the development of veliparib, but also demonstrated the feasibility of conducting proof-of-principle phase 0 trials as part of an alternative paradigm for early drug development in oncology. Pharmacokinetic results from a

phase I study of veliparib in combination with the alkylating agent temozolomide were consistent with those seen in the phase 0 study. While the clinical benefit rate (CBR) and PFS were 17% and 1.9 months, respectively, in patients with heavily pretreated MBC including TNBC, among the BRCA1/2 mutation carriers the CBR was 62% and the median PFS 5.5 months, highlighting the impact of the dysfunctional homologous recombination and synthetic lethality with PARP inhibition [80]. In an expansion cohort of 21 patients, all BRCA mutation carriers, the combination was associated with a CBR of 43% and median PFS of 3.5 months. Several clinical trials of veliparib in the neoadjuvant and metastatic setting are ongoing (supplementary table 5; *www.karger.com/?DOI=455821).*

To date, a major focus of clinical trials in neoadjuvant therapy has been to identify novel regimens that improve the rate of pCR, a finding that might represent a path to approval by the US FDA. To this end, the I-Spy2 trials testing combinations of talazoparib and CPT11, and veliparib and carboplatin have proved a successful example in this setting [81].

Androgen Receptor Antagonists

Gene expression profiling identified a subset of TNBCs with an active hormonally regulated transcriptional program and androgen receptor (AR) expression, and generated interest in targeting the AR [82]. In a phase II single-arm trial of the non-steroidal anti-androgen bicalutamide in patients with TNBC who were AR positive by IHC, over 450 patients were screened, of whom 10% had AR expression; the 6-month CBR with bicalutamide was 19% [83]. It is unclear whether this study provides proof-of-principle, and the modest response could well be due to the indolent nature of luminal disease.

Histone Deacetylase Inhibition

Histone deacetylases (HDACs) are a family of enzymes that regulate chromatin remodeling and gene transcription via the dynamic process of acetylation and deacetylation of core histones. The activity of HDAC inhibitors (HDIs) has been demonstrated in preclinical TNBC models [84, 85]. However, in a randomized phase II study of 62 patients with TNBC, the addition of vorinostat to neoadjuvant carboplatin and nabpaclitaxel was not associated with improved pCR rates [86]. It is recognized that HDIs cause genome-wide effects, specifically they may permit re-expression of ER, or BRCA1/2, in combination with silencing of other genes that have tumor suppressive functions; thus masking a potential antitumor benefit. Efforts are under way to identify which classes of HDACs regulate tumor-promoting classes of genes in order to develop specific therapeutic agents.

Phosphatidylinositide 3-Kinase-AKT-mTOR Pathway Inhibitors

Inhibition of phosphatidylinositide 3-kinase (PI3K) and downstream AKT and mammalian target of rapamycin (mTOR) have been recognized as promising therapeutic targets, due to their known hyperactivation and participation in different tumorigenic processes in numerous malignancies. Activation of the PI3K pathway, either directly via PI3KCA mutations or indirectly via PTEN loss and/or INPP4B loss, is common in TNBC BL breast cancer, as shown in the TCGA initiative [87]. Preclinical studies demonstrated that inhibition of the PI3K pathway results in transient quiescence in TNBC [88]. Preclinical data also support effective inhibition of the PI3K/mTOR pathway in the M and MSL subsets of TNBC [12]. The BL subtype of TNBC has also been shown to be sensitive to mTORC1 inhibitors in vitro and in vivo, again resulting in quiescence [88, 89]. The combination of a PI3K inhibitor with a PARP inhibitor was shown to be synergistic in vivo in an endogenous mouse model for BRCA1-related breast cancers [90]. In TNBCs without BRCA mutations, PI3K blockade results in homologous recombination impairment and sensitization to PARP inhibition. In effect, PI3K inhibition creates a genomic instability similar to that of BRCA1 mutations. These data were the rationale for an ongoing phase I/II clinical trial (NCT01623349) of a PI3K/PARP inhibitor combination in metastatic TNBC [91]. Studies suggest that concomitant CDK4/6 inhibition improves initial responses to PI3K inhibitors and overcomes acquired resistance in PIK3CA mutated breast cancers, providing the rationale for study of this drug combination in PIK3CA mutated breast cancer [92].

Weekly paclitaxel with everolimus followed by anthracycline in a neoadjuvant clinical trial of 50 women with TNBC showed no statistically significant increase in pCR (30% vs. 26%, $p = 0.76$) [93]. A small study of everolimus plus carboplatin in 25 women with advanced TNBC reported a CBR of 36% and a median PFS of 3.3 months, with thrombocytopenia being the most common dose-limiting toxicity requiring carboplatin dose reduction [94].

Mitogen-Activated Protein Kinase Kinase

In TNBC, *Ras* and *Raf* are rarely mutated; however, activation of the mitogen-activated protein kinase (MAPK) pathway is observed and thought to be caused by multiple mechanisms including activation of upstream receptor tyrosine kinases (RTKs), and/or activating mutations in proteins upstream. Suppression of MAPK kinase (MEK) induces a compensatory feedback effect that activates a range of upstream RTKs [95]. A novel-design preoperative clinical study indicated that the pattern of feedback is dictated by molecular phenotype and is different between BL versus M TNBC. Importantly, both subtypes share a subset of kinases enabling identification of potential pathways of acute resistance and suggesting rational combinations with MEK inhibitors [96, 97].

One of the pathways known to be activated in response to MEK inhibition is PI3K/Akt [98], which has led to a large number of preclinical studies evaluating the efficacy of MEK inhibitors in combination with PI3K/mTOR pathway inhibitors in TNBC [99] and other malignancies [100, 101]. More precise characterization of the antitumor effects of different combinations of MEK with PI3K pathway inhibitors in the BL and M subtypes of TNBC is anticipated [102].

Checkpoint Kinase 1 Inhibition

Checkpoint kinase 1 (Chk1) inhibitors have become an attractive potential target for the treatment of TNBC harboring p53 mu-

tations. In addition to the TCGA initiative, several studies have identified high rates of p53 mutations in TNBC. In this scenario cells in need of DNA-damage repair rely on Chk1 to arrest the cell cycle and push potentially defective cells toward apoptosis. Also, p53-deficient mouse models of breast cancer were shown to be sensitive to Chk1 inhibition [103, 104].

Immune Modulation

The belief of a nonimmunogenic status in breast cancer has long been debated, presenting a limiting factor in evaluating the effect of immunotherapy. Robust data recently suggested that breast cancer and particularly HER2-positive and triple-negative tumors are in fact immunogenic and that the extent of immune response correlates with effectiveness. Results from recent studies suggest potential value of immune modulation in treating breast cancer patients with an aggressive biology, and support the value of immunotherapy in TNBC. Gene expression profiling demonstrated an association between expression of immunomodulatory genes and better clinical outcomes in TNBC. The presence of immune cells in the breast cancer microenvironment has long been recognized as a good prognostic factor. At diagnosis, only approximately 5–15% of TNBCs are classified as lymphocyte predominant, defined as either > 50% or > 60% lymphocytes in the stroma, a further 15–20% of TNBCs has no lymphocytic infiltration, while the majority of cases 65–80% harbor low to moderate level of immune cells [105]. Tumor infiltrating lymphocytes (TILs) were reported to be prognostic and also predictive in TNBC.

With regard to prognosis, Loi et al. [106] showed using primary tumor samples from the BIG 02–98 study that every 10% increase in intramural (iTILs) and stromal TILs (sTILs) was associated with 17% and 15% reduced risk of relapse ($p = 0.10$ and $p = 0.0025$), respectively, and 27% and 17% reduced risk of death ($p = 0.035$ and $p = 0.023$), respectively, in patients with TNBC. Similar results were reported from the combined analysis of adjuvant trial E2197 and E1199. sTILs positively correlated with distant recurrence-free survival and OS [107].

With regard to prediction, several neoadjuvant studies demonstrated significantly higher pCR rates among immune-rich compared to immune-poor TNBCs. A prospective analysis in the GeparSixto trial showed that 60% of 142 patients with lymphocyte predominant breast cancer achieved pCR compared with 40% of all the women in the study, and 34% of women with low levels of TILs ($p < 0.0005$). The predictive effect for response to NAC was particularly high in patients treated with carboplatin. In fact, a 74% pCR was reported for lymphocyte predominant TNBC patients treated with carboplatin plus paclitaxel/doxorubicin [108]. In the PreCOG 0105 study, both sTILs and iTILs were shown to be predictive of response to platinum-based neoadjuvant therapy and were associated with the IM subtype [109]. Lymphocyte predominance in residual cancer (≥ 60% of stromal cells) after NAC, which is seen in a small minority (≤ 10%) of TNBC treated with NAC, is also associated with excellent survival even in patients who have high-risk pathological features such as positive nodes or > 2-cm residual tumor size [110, 111].

Several immunotherapy strategies, aiming at blocking or activating specific targets, are currently under study, including antagonists against inhibitory up-regulated receptors on antitumor T cells, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) [112]. Recent evidence for specific tumor antigens, such as MUC-1 and NY-ESO-1, led to the development of targetable vaccine antigens. Early phase trials targeting the T-cell inhibitory molecule programmed death-ligand 1 (PD-L1) have shown clinical efficacy in cancer [113]. The TCGA RNA sequencing data showed significantly greater expression of the PD-L1 gene in TNBC compared to non-TNBC [87], and a recent report showed an association of biomarkers involved in immune evasion, including PD-L1 with other biological pathways. In newly diagnosed early stage breast cancer, PD-L1 is expressed in approximately 20–30% of cases, and primarily in TNBC [114] is associated with TILs [115] and correlates with higher histological grade [116]. Results suggest that subsets of TNBC, such as AR-negative TBNC, might derive benefit from PD-L1- and CTLA-4-targeted therapy. Mutations or deletions in the PTEN/PI3K pathway have been implicated in breast cancer, and / or loss of PTEN, particularly in hormone receptor-negative breast cancer [117 leads to the upregulation of PD-L1 and suppression of T-cell proliferation and survival [114]. The positive correlation of PIK3CA and PD-L1 also indicates that combination therapy targeting both pathways may be beneficial [118]. In addition, the inverse correlation of BRCA1 status with PD-L1 suggests a potential role for platinum-based therapy in combination with anti-PD-L1. Further prospective validation of these findings is ongoing [119].

The safety and antitumor activity of the PD-1 inhibitor MK 3475, pembrolizumab, was investigated in a phase Ib study (KEYNOTE-012) of 32 female patients with PD-L1-positive advanced TNBC. In the 27 evaluable patients, preliminary evidence of clinical activity with an overall response rate of 18.5% and median time to progression of 18 weeks, median duration of response was not yet reached and a potentially acceptable safety profile of pembrolizumab was reported [120]. It is notable that in this study the ORR associated with single-agent pembrolizumab was approximately double that reported for capecitabine (9%) as a second or higher line therapy for triple-negative disease in a prespecified subgroup analysis of a phase III clinical trial [121]. A single-agent phase II study examining a 200-mg dose given once every 3 weeks is ongoing (clinical trials.gov identifier: NCT02447003).

The second phase I trial (NCT01375842) tested the efficacy and safety of the anti-PD-L1 antibody atezolizumab (MPDL3280A) and required ≥ 5% PD-L1 positivity by IHC [122]. 69% of patients who were screened tested positive for PD-L1 expression. 3 dose levels were evaluated: 15 mg/kg, 20 mg/kg and 1,200 mg fixed dose. 21 patients were evaluable for efficacy, and a 19% ORR was observed; the 24-week PFS was 27%. 54 patients were evaluable for toxicity: most adverse events were grade 2 or lower, but 11% had treatment-related grade 3 or higher adverse events. Overall, these response rates are similar to those seen with single-agent chemotherapy as second or third line treatment for

metastatic TNBC, but appear to be more durable. Combination studies of pembrolizumab with other anticancer therapies are in development.

Combining Radiation and Immune Checkpoint Inhibition

Cases documenting an abscopal effect of radiation have been reported for more than 40 years [123]. Recent preclinical and clinical data suggest that localized radiotherapy can also induce, enhance and/or modulate tumor-associated immune response [124]. However, the molecular determinants of that response are not clear. Direct action of radiation on the tumor is immunogenic, releasing tumor antigens as well as other activating factors. Radiation directly activates cell-mediated immunity at 1 or more critical regulatory points. Irradiating tumors can provide a source of tumor-specific antigen, which in turn can evoke an antigen-specific immune response. It can also augment the immune response through the tumor microenvironment by various types of inhibitory immune cells that can suppress T cell activation and promote tumor growth. In a preclinical model, local radiation increased the expression of PD-L1 on dendritic cells. This local upregulation of the PD-L1-PD-1 axis following radiation suppresses radiation-induced immune responses. Treatment with combined radiation plus anti-PDL-1 effectively controlled tumor growth [6].

Conclusion

At present, treatment options for TNBC remain limited both in number and efficacy; however, there is considerable research focusing on the identification and elucidation of 'drugable' targets and pathways that underlie the aggressive biology of this heterogeneous disease. Concerted efforts in this area will ensure the emergence of novel strategies in the management of TNBC, with the ultimate long-term goal of replacing nonspecific standard of care therapy with rationally-derived treatment regimens.

The era of genomics has enabled a paradigm-shifting reclassification of TNBC that reveals a spectrum of previously uncharacterized genetic lesions that rationalize the basis for the recalcitrant nature of the disease, which is profound molecular heterogeneity.

Studies of BRCA1 mutated breast cancers indicate sensitivity to DNA-damaging chemotherapeutics such as cisplatin or carboplatin, as well as to PARP inhibitors alone or in combination with DNA damaging agents. Inhibitors of the PI3K/AKT/mTOR pathway, commonly deregulated in TNBC, are also being investigated.

The CALGB 40603 investigators, the Alliance Breast Committee, and NCCN have not endorsed the use of platinum agents as a new standard of care for patients with TNBC. The impact of platinum agents on DFS and OS and the potential benefits of additional therapy in the post-neoadjuvant setting are considered to be among the most pressing clinical questions in current practice.

Lymphocytic infiltration is associated with improved response to neoadjuvant chemotherapy and better prognosis in TNBC, raising the possibility of employing immune checkpoint blockage as a therapeutic strategy.

Supplementary Material

Supplementary Table 1. Summary of clinical and molecular characteristics of TNBC

Supplementary Table 2. Novel agents in clinical development for treatment of TNBC

Supplementary Table 3. Revised Molecular Subtype of TNBC (TNBCtype-4)

Supplementary Table 4. Platinum Trials in the Neoadjuvant Setting in TNBC and BRCA-Associated Breast Cancer

Supplementary Table 5. Selected active clinical trials for TNBC or BRCA-associated breast cancer

Supplementary Fig. 1. Cancer Systems Biology: embracing complexity to develop better anticancer therapeutic strategies.

Supplementary Fig. 2. Residual Cancer Burden (RCB) Index, a measure of the residual tumor burden after neoadjuvant chemotherapy, predicts risk of distant relapse at five years.

To access the supplementary figures and tables as well as the references, please refer to *www.karger.com/?DOI=455821*.

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References

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