Emerging targeted therapies in triple-negative breast cancer

J. Crown¹,²*, J. O’Shaughnessy³ & G. Gullo¹

¹St Vincent’s University Hospital, Dublin, Ireland; ²Molecular Therapeutics for Cancer Ireland, NICB Dublin City University, Dublin, Ireland; ³Baylor Charles A. Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, USA

Standard chemotherapy regimens can prove effective for patients with early triple-negative breast cancer (TNBC); however, patients with advanced disease typically respond poorly and rapidly progress, and the outcome is poor. New targeted therapies are therefore an urgent unmet medical need for this patient population. Translational and clinical studies into new TNBC treatments have been facilitated by the increased understanding of the aberrant signal transduction pathways regulating growth and survival and the development of chemoresistance in TNBC. Some of the established targeted agents that have been approved in other indications may prove beneficial to patients with TNBC; however, in the absence of approved targeted agents for the treatment of TNBC, most new agents remain experimental. Increased understanding of molecular profiles of TNBC subtypes is likely to improve therapeutic strategies with targeted agents. Novel strategies have reached clinical evaluation in patients with TNBC, including targeting angiogenesis vascular endothelial growth factor and proliferation signalling (receptor tyrosine kinases and mammalian target of rapamycin). Aggressive TNBCs have been found to associate closely with BRCA1 mutation or dysregulation. The recent development of new investigational agents targeting DNA repair, either directly with poly(adenosine diphosphate-ribose) polymerase inhibitors or indirectly through DNA-binding or DNA-damage potentiation, is a major focus of current clinical studies. These and other targeted therapies represent a new approach to TNBC therapy.

Key words: targeted, therapies, triple-negative breast cancer

introduction

Although the term ‘triple-negative breast cancer’ (TNBC) appears to have been first mentioned in the medical literature no longer than 6 years ago [1], it has achieved such a degree of scientific interest that the TNBC category is now fully incorporated into the oncology glossary. Today TNBC is probably one of the most active areas of research in oncology. The reasons for this scientific interest are easy to understand: first, the lack of a recognised target for molecular-oriented therapy in the general context of current therapeutic approaches to breast cancer makes TNBC a new orphan disease. Second, the relatively poor prognosis of patients with TNBC, especially those with advanced-stage disease, makes TNBC an extremely challenging and frustrating condition for both medical oncologists and patients.

The challenges of TNBC are in fact more fundamental than insensitivity to current available therapeutics. They originate from the characteristics of triple-negativity for breast cancer. This is a substantially histopathological category based, by definition, on the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and without alteration of the human epidermal growth factor receptor type 2 (HER2) gene. However, when this histopathological category is matched with the gene expression analysis, it appears very clear that TNBC is a heterogeneous subtype of breast cancer, which shows only a partial overlapping with the so-called basal-like breast cancer. In fact, distinct from TNBC, the basal-like breast cancer subtype is a molecular category showing substantial similarities to the basal/myoepithelial cells of the normal breast. Approximately 80% of TNBCs fall into the basal-like breast cancer subtype based on gene expression analysis [2], but TNBC also encompasses other molecular subtypes of breast cancer, such as the claudin-low subtype. Many efforts have been made to standardise a method to distinguish between basal and non-basal subtypes within TNBC, but no consensus has been agreed to date [3, 4]. Although there is as yet no clinical utility in understanding whether a TNBC is of the basal-like or other intrinsic subtype, TNBC and basal-like breast cancer should never be considered as synonymous and the two categories should always be kept separated for either clinical or research purposes.

It should be noted that there is still no uniformity across studies regarding the definition of triple-negativity based on immunohistochemistry (IHC), particularly with regard to ER and PR status. Some investigators consider breast tumours as being negative for the expression of ER or PR if <1% of cells show immunoreactivity for ER or PR, whereas others set the
cut-off for ER or PR positivity at 10% of immunoreactive tumour cells. The discrepancies in ER, PR and HER2 testing among studies can represent a further problem when interpreting results or applying data to clinical practice. Recommendations for ER and PR IHC testing have been recently updated by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), with ER- and PR-negative tumours now being defined in ASCO–CAP Guidelines as having <1% of cells positive for either receptor by IHC [5].

**Chemotherapy**

As TNBC cannot be treated with either hormonal therapy or anti-HER2 agents, standard chemotherapy is the backbone of systemic treatment. There is a consensus that TNBC shows increased chemosensitivity compared with ER-positive breast cancer, although no optimal cytotoxic regimen has been identified. Patients with TNBC who achieve a pathological complete response (pCR) after neoadjuvant chemotherapy show survival rates equivalent to those of patients with other breast cancer subtypes [6, 7]. Despite this, the overall prognosis for patients with TNBC is poorer than for those patients with a non-TNBC phenotype. This observation is likely explained because the majority of patients with TNBC have residual disease in the breast and lymph nodes after pre-operative chemotherapy. With no targeted treatments currently available, patients with TNBC have a high risk of relapse and a shorter overall survival (OS) compared with other breast cancer subtypes.

The most common chemotherapeutic approach to advanced TNBC is based on anthracycline and taxane combinations for the first line of treatment, followed by capecitabine at the time of progression [8]. In the last few years, there has also been increased use of platinum-derived agents based on the platinum sensitivity shown by *BRCA*-1-mutated breast cancers, which typically have a triple-negative phenotype. However, there is little prospective evidence from random assignment trials to support the preferred use of platinum compounds over other more standard cytotoxic agents for TNBC, especially for early-stage disease. Most recent data on TNBC are based on the use of carboplatin, but data from older studies suggest that cisplatin might be a more active agent against breast cancer [9–20].

Newer chemotherapeutic agents include the epothilone B analogue ixabepilone, a microtubule-targeting drug which is now US Food and Drug Administration (FDA)-approved for advanced breast cancer in combination with capecitabine after failure of an anthracycline and a taxane [21]. A pooled analysis of patients with TNBC from two large phase III clinical trials showed that the addition of ixabepilone to capecitabine resulted in an approximately twofold increase in median progression-free survival (PFS) versus capecitabine alone (from 1.7 to 4.2 months), and that objective responses to ixabepilone in TNBC patients were comparable with those seen in patients with non-triple-negative tumours [22]. No substantial difference in OS between ixabepilone/capecitabine over capecitabine alone has been observed so far [23]. A recent three-arm phase II study has shown the activity and tolerability of ixabepilone plus bevacizumab; however, comparative data are not yet available [24]. These results warrant further investigation of ixabepilone in patients with TNBC, and several clinical trials are currently ongoing in this population.

Eribulin has recently been granted approval by the US FDA for advanced breast cancer patients who have received at least two chemotherapeutic regimens, which included an anthracycline and a taxane. Approval was based on the results of the phase III clinical study (EMBRACE) of single-agent eribulin versus treatment of physician’s choice (TPC), which demonstrated a statistically significant increase in OS compared with TPC [median OS 13.1 versus 10.6 months, hazard ratio (HR) 0.81, *P* = 0.041]. The majority (74%) of patients enrolled in the trial were HER2-negative and 19% had TNBC [25]. Subgroup analyses from the EMBRACE study indicated that eribulin was generally more effective in ER-negative patients, who had a 34% decreased risk of death compared with TPC, and in patients with TNBC, who had a 29% risk of death reduction. Of note, eribulin was less effective in patients who were not pretreated with capecitabine [26].

Overall, standard chemotherapy regimens can prove effective for a subgroup of patients with early chemosensitive TNBC. However, patients with advanced disease typically respond poorly to current chemotherapeutic agents. Where a response is seen, patients subsequently show rapid disease progression. New, more targeted therapies are therefore an urgent unmet medical need for this population of patients with TNBC, regardless of the stage of disease at diagnosis.

**Targeted Therapies in TNBC Treatment**

It will be difficult to develop new effective targeted agents for TNBC without a clear understanding of the molecular pathology of TNBC, including the pathogenesis of disease and mechanisms of resistance to current available therapies. In the last few years, there have been insights into the role that aberrant signal transduction pathways may play in regulating growth and survival and in developing chemoresistance in TNBC. Overexpressed proteins in TNBC may provide future therapeutic targets and include epidermal growth factor receptor (EGFR), c-kit, cytokeratins 5, 14 and 17, p53, p15 and cyclin E.

The molecular pathways currently under investigation in TNBC are presented in Figure 1, together with the proteins identified as potential therapeutic targets. Some of the established targeted agents that have shown substantial clinical activity in other malignancies are being investigated in patients with TNBC. With the only exception of bevacizumab, they remain experimental and are therefore not available outside the clinical trial setting.

**Anti-VEGF/VEGFR Monoclonal Antibodies**

TNBC is a highly proliferative neoplasm that needs constant angiogenesis throughout all the phases of its development, invasion and metastasis [27]. The anti-vascular endothelial growth factor (VEGF) monoclonal antibody, bevacizumab, has been shown to increase response rate (RR) and PFS of patients with metastatic breast cancer when added to first-line
chemotherapy in three randomised phase III trials [28–31], although its approval for use in metastatic breast cancer has now been rescinded by the regulatory authorities in the United States. None the less, a subgroup analysis of the E2100 trial of paclitaxel alone or with bevacizumab showed advantages for the addition of bevacizumab to standard paclitaxel in the TNBC subgroup [28]. A subgroup analysis of the TNBC population in the MO19391 observational (non-randomised) study of bevacizumab in combination with taxane-based therapy or other non-anthracycline-containing regimens found an RR of 47% and a median time-to-progression of 7.1 months. Of note, they were both inferior to those observed in the non-TNBC population from the same study [29]. The recently published results of the RIBBON-1 trial indicated that bevacizumab may be beneficial when added to standard chemotherapy of capecitabine or anthracycline/taxane regimens in the TNBC patient subset [30]. In a subgroup analysis of the RIBBON-2 trial, various chemotherapies were investigated with and without bevacizumab as second-line treatment of metastatic breast cancer. In patients with TNBC, improvements in PFS with bevacizumab were marked (median 6.0 versus 2.7 months for chemotherapy alone; \( P = 0.0006 \)) with a trend towards improved OS [31].

Bevacizumab has also been investigated in the neoadjuvant setting, and data from two large randomised clinical trials in patients with HER2-negative operable breast cancer have recently been presented. In the Gepar-Quinto trial [32], the addition of bevacizumab to standard preoperative chemotherapy with a sequential anthracycline/taxane regimen produced a substantially higher rate of pCR in the TNBC subgroup, although no data on long-term follow-up are available yet [32]. However, these results were not confirmed in the NSABP B-40 study, in which bevacizumab had only a minimal effect in patients with TNBC [33]. The differences in the results from the two trials could be attributed to differences in how HER2-negativity was defined in the patient groups treated in the trials and in the therapy regimens they received. In the Gepar-Quinto trial Gerber et al. [32] defined TNBC using HER2 IHC score 0/1 (or FISH-negative; detected locally), whereas Bear et al. in the NSABP B-40 trial [33] defined the TNBC subpopulation using HER2-negative confirmed by IHC (<3+) or by FISH (negative for gene amplification). Furthermore, Gerber et al. [32] added bevacizumab to a sequential epirubicin/cyclophosphamide/docetaxel therapy regimen, whereas patients in the study conducted by Bear et al. [33] received one of three taxane-based chemotherapy regimens with or without bevacizumab. In both trials, the addition of bevacizumab to anthracycline/taxane chemotherapy led to an increase in overall toxicity of treatment, especially hypertension, mucositis and complicated wound healing. Bevacizumab is also currently under investigation in a large phase III clinical study as adjuvant therapy in addition to chemotherapy for TNBC patients (ClinicalTrials.gov NCT00528567).

Bevacizumab binds specifically to the ligand, vascular endothelial growth factor-A (VEGF-A) and all its isoforms, but has limited binding affinity for other VEGF receptor (VEGFR) ligands VEGF-B and VEGF-C [34]. In contrast, newer anti-angiogenic agents bind to VEGFR-2 and thus prevent all ligand binding to this target. Targeting VEGFR in this way
could potentially lead to a more complete target inhibition and a block angiogenesis more effectively. The monoclonal antibody ramucirumab (IMC-1121B, ImClone) targeting VEGFR-2 is currently under investigation in combination with docetaxel in a phase III clinical trial in patients with HER2-negative metastatic breast cancer (ClinicalTrials.gov NCT00703326).

### anti-VEGFR tyrosine kinase inhibitors

The anti-VEGFR tyrosine kinase inhibitors (TKIs) sunitinib and sorafenib have also shown some interesting degrees of activity in breast cancer in clinical studies with substantial TNBC populations [35, 36]. Sunitinib at a dose of 50 mg/day was found to be active in a phase II clinical trial as a single agent in heavily pretreated patients with metastatic breast cancer [35]. These results led to the design of several phase III trials to define a role for sunitinib in HER2-negative advanced breast cancer. A trial comparing head-to-head single-agent sunitinib (37.5 mg continuous daily dosing) with capecitabine in patients with HER2-negative advanced breast cancer pretreated with anthracycline and taxane was terminated early after a first interim analysis that indicated that PFS was shorter with sunitinib than with capecitabine (median 2.8 versus 4.2 months) [37]. No details on the TNBC population are available from this trial. Bianchi et al. [38] describe the results of single-agent sorafenib in metastatic breast cancer. This study included 56 patients and demonstrated a 2% RR and 13% stable disease at 6 months. The authors concluded that further investigation of single-agent sorafenib in this patient population is not recommended.

The addition of sunitinib to first- and second-line chemotherapy, with docetaxel and with capcitabine, respectively, was investigated in two large phase III clinical trials in patients with HER2-negative metastatic breast cancer [39, 40]. Neither of these studies met their primary objectives, and neither showed benefit from the addition of sunitinib to standard first- or second-line chemotherapy. It is uncertain whether sunitinib will be investigated further as a single-agent, or in combination with chemotherapy, in HER2-negative advanced breast cancer. The different dosing and schedule of sunitinib across the trials might at least in part explain the results, but the lack of tissue collection will not allow for any additional translational exploratory analysis in any subgroup of patients, including TNBC.

Sorafenib was found to extend median PFS when added to capcitabine chemotherapy in a phase II clinical trial in HER2-negative patients pretreated with no more than two lines of chemotherapy for metastatic disease [36]. This study did not stratify for ER or PR status, and therefore some patients had TNBC tumours, while others were HER2-non-overexpressing and hormone receptor-positive. The positive effect that an ER-positive status brings to patients with breast cancer must be taken into consideration here. Patients who achieve pCR have a better outcome than those who do not, irrespective of the tumour subtype. Where residual disease remains, it has been shown that an ER-positive status predicts for better disease-free survival [41]. A phase III confirmatory trial is ongoing to verify the findings in this study. There is additional concern surrounding the toxicity of sorafenib and its effects when combined with other cytotoxic therapy regimens. In a recent feasibility study of sorafenib in combination with paclitaxel [42], which involved 45 patients with node-positive or high-risk early-stage breast cancer, 47% of patients stopped treatment due to the toxic effects of the treatment received.

New small-molecule receptor TKIs such as apatinib (YN968D1, anti-VEGFR, monotherapy) and cediranib (in combination with olaparib) have reached phase II clinical development in patients with TNBC.

### anti-EGFR therapies

A large proportion of TNBC tumours overexpress EGFR, which is also a negative prognostic factor in TNBC [4, 43]. Therefore, EGFR might be an attractive therapeutic target in TNBC, and many anti-EGFR agents are already available in clinical practice for use in other malignancies. The monoclonal antibody cetuximab has shown some interesting activity in patients with TNBC when combined with single-agent carboplatin [objective response rate (ORR) = 18%] [44] and with carboplatin/irinotecan (ORR = 49% in the TNBC population) [45]. The use of platinum agents is becoming increasingly common in TNBC, which is related to the inherent nature of these tumours. Over 50% of TNBCs are basal-like breast cancers and are believed to have defects in homologous recombination, or repair of double-strand DNA breaks. Platinum-based therapies introduce DNA damage, hence patients with BRCA1 mutations or, it is hypothesised, basal-like TNBC may have an increased sensitivity to platinum-based drugs compared with other breast cancer subtypes [8]. This was the rationale for the evaluation of cetuximab with cisplatin in a randomised phase III trial (BALI). Overall, 173 patients with metastatic TNBC, who had received no more than one previous line of chemotherapy for metastatic disease, were given cisplatin, with or without cetuximab. The addition of cetuximab resulted in a modest improvement in activity (ORR 20% versus 10.3%; PFS 3.7 versus 1.5 months) [46]. Although the RR was doubled in the combination arm, it did not meet the pre-specified primary end point of the study (up to an RR of 32%) and was not found to be statistically significant. Efforts are under way to identify those patients with metastatic TNBC who benefit from cetuximab treatment, which may be correlated with lower expression of alpha-crystallin B chain (encoded by the CRYAB gene), higher expression of phosphatase and tensin (PTEN) homologue and lack of KRAS expression in patients with basal-like breast cancer [47].

The cetuximab-related compound panitumumab is currently being investigated in a phase II trial in combination with carboplatin and paclitaxel in patients with metastatic HER2-negative breast cancer with ER and PR expression of <10% (ClinicalTrials.gov NCT01009983). Another phase II trial with panitumumab, gemcitabine and carboplatin in patients with metastatic TNBC has recently suspended recruitment (ClinicalTrials.gov NCT00894504). The suspension of this trial was due to funding issues and was not related to safety or efficacy results.
Small-molecule TKIs against EGFR are also being evaluated in TNBC. Baselga et al. [48] reported that gefitinib showed minimal single-agent activity in patients with metastatic breast cancer—this was consistent with the results of a study by Dickler et al. [49], which concluded that erlotinib also showed minimal activity as a single-agent therapy for patients with metastatic breast cancer. These studies, however, included patients with metastatic breast cancer who were not selected for the presence of activating mutations in the EGFR gene. In other tumour types, stratifying the patient population according to EGFR activation status has improved RRs with EGFR TKIs. Methodological difficulties, however, can hamper the accurate assessment of EGFR mutational status (to ensure the determination of activated versus total EGFR expression) [50]. It remains unknown whether EGFR TKIs add any value to the therapy for TNBC [43, 51]. However, preclinical data support the use of erlotinib or gefitinib combined with docetaxel or carboplatin in TNBC cell lines [43]. Furthermore, erlotinib is currently being evaluated in TNBC in several phase I and II clinical trials, in combination with various chemotherapy regimens. Newer EGFR TKIs are also in development, including neratinib, which is being evaluated in a phase I/II clinical trial in combination with the mTOR Complex 1 (mTORC1) inhibitor temsirolimus in patients with trastuzumab-refractory metastatic HER2-amplified breast cancer or TNBC. The recently reported phase I data demonstrated an RR of 67%, and the combination was well-tolerated [52].

PARP inhibitors
Genomic integrity and cell survival are critically dependent on coordinated pathways of DNA repair [50]. Poly(adenosine diphosphate-ribose) polymerase (PARP) enzymes, particularly the most abundant isoform PARP1, play a key role in these pathways by mediating the repair of single-strand DNA breaks via base excision repair [53]. Consequently, loss of PARP activity results in the accumulation of single-strand breaks, which are normally repaired by double-strand homologous recombination pathways that include the important tumour-suppressor proteins BRCA1 and BRCA2 [54]. It is well-known that germ-line BRCA1 and BRCA2 mutations are associated with a high risk of oncogenesis for breast and ovarian cancers [55]. TNBC shares clinical and pathological features with hereditary BRCA1-related breast cancers, and in sporadic TNBC, dysregulation of BRCA1 has been frequently observed together with other defects in homologous recombination pathways [56, 57]. Preclinical studies have shown that breast cancer cell lines with a triple-negative phenotype are more sensitive to PARP1 inhibitors compared with non-TNBC cells, and that PARP inhibition synergises with gemcitabine and cisplatin in triple-negative cells but not in luminal cancers [58]. All these lines of evidence provide a strong rationale for developing a new therapeutic approach to TNBC based on targeting the DNA-repair defects via PARP inhibition in these cancers.

Olaparib is an oral PARP inhibitor in advanced-phase clinical development, which has been investigated as a single agent against BRCA1- and BRCA2-mutated advanced breast cancers in a phase II trial [59]. In this non-randomised trial, the ORR for patients receiving olaparib at the maximum tolerated dose of 400 mg twice daily was higher than that in the cohort of those receiving olaparib at a lower dose of 100 mg twice daily (41% versus 22%). Olaparib toxicity was generally mild and manageable, with the most frequent adverse events in the cohort given 400 mg twice daily being fatigue, nausea, vomiting and anaemia. The results of this study provide an important proof-of-concept for PARP inhibition in BRCA-deficient breast cancers, and show a favourable therapeutic index for a novel targeted treatment strategy in patients with tumours that have genetic loss of function of BRCA1- or BRCA2-associated DNA repair. Selecting patients with TNBC who are also BRCA-deficient would isolate a patient population who may be more receptive to treatment with PARP inhibitors. It is interesting to observe that there is no reported activity for olaparib against non-BRCA-mutant solid tumours or in patients with unreported BRCA status, either as a single agent or in combination with dacarbazine in a phase I study [60, 61].

Velparib (ABT-888), an oral PARP1 and PARP2 inhibitor, has demonstrated preliminary activity in a small single-arm phase II study when combined with temozolomide, in patients with metastatic breast cancer, including TNBC patients, with further studies ongoing [62]. Several other PARP inhibitors, such as MK-4827 (Merck) and PF-01367338 (Pfizer), are currently the subjects of early development in TNBC or BRCA-mutated breast cancers.

Establishing which subtypes of breast cancer are most indicated for PARP inhibitor treatment, and how to identify them, is a matter of utmost importance for further clinical development of this class of anticancer agents. Despite their extremely high therapeutic potential, more research on the biology of the numerous members of the PARP families and on their role in the molecular pathogenesis of BRCA1- and BRCA2-mutated versus TNBC is necessary before we can claim to understand the utility of the PARP inhibitors in clinical oncology.

iniparib
The investigational anticancer agent iniparib was initially developed as a PARP inhibitor, but recent data suggest that it does not possess characteristics typical of this class, and its exact mechanism of action remains to be elucidated. The cellular effects of iniparib include the induction of γ-H2AX foci (a marker of DNA damage) and cell-cycle arrest in the G2/M phase in tumour cell lines [63, 64]. Iniparib also potentiates the cell-cycle effects of DNA damaging modalities in tumour cell lines, and antiproliferative activity has been demonstrated in TNBC-related cell lines [64, 65]. A phase II randomised clinical trial of carboplatin and gemcitabine chemotherapy with or without iniparib in patients with pretreated TNBC showed very promising results, with an increase in ORR (52% versus 32%, P = 0.02), PFS (5.9 versus 3.6 months, HR 0.59, P = 0.01) and median OS (post hoc evaluation 12.3 versus 7.7 months, HR 0.57, P = 0.01) [66]. Of note, toxicity of iniparib was extremely mild and did not increase substantially the adverse events of the carboplatin/
gemcitabine chemotherapy. The recently presented results of a confirmatory phase III study in 519 patients with substantially identical design, treatment regimen and schedule failed to confirm the findings from the phase II trial, and no difference in PFS or OS was observed [67]. Whether patient selection factors such as disease-free interval may have differed between the phase II and III populations is being investigated. Nonetheless, a prespecified subset analysis suggested a possible efficacy benefit among patients receiving second- or third-line chemotherapy plus iniparib (222 patients) compared with patients who had not received prior chemotherapy for metastatic TNBC (first-line subset, 297 patients). Biomarker analyses on archival primary breast cancer tissue are under way to identify whether specific biological subsets of patients with TNBC may benefit from iniparib treatment.

**mTOR inhibitors**

The mammalian target of rapamycin (mTOR) is an effector of the phosphoinositide 3-kinase (PI3K) signalling pathway regulated by AKT and the tumour-suppressor PTEN. Proteins belonging to the PI3K pathway are frequently affected by mutations in breast carcinomas, and loss of PTEN is a common finding in TNBC, thus leading to increased mTOR activation in the disease [68]. This represents an interesting rationale for testing mTOR inhibitors in TNBC, and currently two mTOR inhibitors are being evaluated in patients with HER2-negative breast cancer or TNBC.

In a phase II study of the first- or second-line treatment of patients with metastatic breast cancer, oral everolimus (RAD001) at a dose of 10 mg/day gave an ORR of 12%. No objective response was observed at the dose of 70 mg/week [69]. The most common drug-related adverse events were fatigue, rash, anorexia, diarrhoea, stomatitis, cough and pneumonitis. Pneumonitis occurred more frequently than expected in this trial and appeared to be schedule-dependent, with the highest incidence on the daily schedule. No biological correlates of response could be identified in this study, although there were trends favouring benefit in the ER-positive and HER2-negative population. Several clinical phase I and II trials of everolimus alone or in combination with other agents such as lapatinib (ClinicalTrials.gov NCT01272141), carboplatin (ClinicalTrials.gov NCT01127763) and neoadjuvant cisplatin/paclitaxel (ClinicalTrials.gov NCT00930930) are currently recruiting patients with TNBC. Results are also awaited from a phase II (N = 110) randomised, double-blind, placebo-controlled trial evaluating the impact of adding everolimus to the combination of weekly paclitaxel plus bevacizumab in the first-line treatment of women with HER2-negative metastatic breast cancer (ClinicalTrials.gov NCT00915603). The mTOR inhibitor temsirolimus is also being investigated in a phase I/II clinical trial in combination with neratinib (ClinicalTrials.gov NCT0111825) in metastatic TNBC and in a phase III randomised neoadjuvant study in combination with an anthracycline and a taxane.

A number of studies in breast cancer patients are currently investigating PI3K inhibitors. For example, BEZ235, a dual PI3K/mTOR inhibitor, is being studied alone or in combination with the MEK inhibitor MEK162 in several phase I/II clinical studies on types of cancer that also include TNBC.

**Src tyrosine kinase inhibitor**

Src tyrosine kinase (Rous sarcoma virus) is overexpressed in TNBC, and has been associated with metastatic disease progression [70]. Dasatinib, an oral inhibitor of Src, c-kit and PDGFRα, inhibits the growth of basal-like breast cancer and TNBC cell lines, and was synergistic when combined with cisplatin in three dasatinib-sensitive cell lines [70, 71]. Single-agent dasatinib was evaluated in patients with metastatic TNBC pretreated with an anthracycline and a taxane in a single-arm phase II study. In this trial, dasatinib at a dose of 70 mg twice daily produced a disease control rate (objective response + stable disease for at least 16 weeks) of 9.3%, with a 4.3% confirmed partial RR and median PFS of 8.3 weeks [72]. A recently published phase I/II study of dasatinib and weekly paclitaxel showed preliminary activity (including in patients previously treated with taxanes) with a manageable toxicity profile [73].

**other classes of targeted agents**

Translational and clinical studies into new TNBC-targeted treatments strictly depend on an increased understanding of the molecular pathology of TNBC, and ongoing research is currently exploring the possibility of inhibiting proliferation via mechanisms or pathways other than those described previously. Of note, most of these alternative potential therapeutic strategies have just started to emerge, and presently no advanced clinical data are available. These strategies include targeting epigenetic modifications of breast cancer with histone deacetylase inhibitors such as vorinostat and trichostatin A, although no current studies specifically target the TNBC population. The notch/secretase inhibitor RO4929097 is also currently under investigation in combination with neoadjuvant chemotherapy with paclitaxel and carboplatin in a phase I trial for operable TNBC (ClinicalTrials.gov NCT01238133). The marine natural product DNA-damaging agent trabectedin, is the subject of a phase II clinical trial in patients with metastatic breast cancer pretreated with anthracyclines/taxanes that has recently completed accrual (ClinicalTrials.gov NCT00050427) and of a phase II trial in patients with several subtypes of breast cancer including the triple-negative subgroup (ClinicalTrials.gov NCT00580112).

The heat-shock protein 90 (HSP90) chaperone protein is important in post-translational maturation of oncogenic client proteins, including some gene products with a well-recognised role in maintaining aberrant signal transduction pathways such as AKT, CDK4 and androgen receptors [74]. The HSP90 inhibitor PU-H71 showed potent and durable antitumour effects in TNBC xenografts, including complete response and tumour regression [75], thus appearing to have a potential therapeutic role in this subgroup of breast cancer.

Finally, a recent study has identified the interleukin-6/Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signalling pathway in CD44+/CD24− stem cell-like breast cancer cells, as a potential therapeutic
target, particularly in basal-like breast cancer [76]. The JAK1 and JAK2 inhibitor ruxolitinib is currently being investigated in a number of human malignancies, including solid tumours [77].

**conclusions and future directions**

There have been striking advances in breast cancer therapy over the last few years; however, much of this progress has been confined to the subset of patients who overexpress HER2. TNBC is defined by the absence of a target, so that today, triple-negativity is only a label applied to the heterogeneous group of all breast tumours that do not express ER, PR and HER2. The ability to employ a tailored therapeutic approach will be limited without the characterisation of specific markers for this breast cancer subtype. Such markers will allow novel targeted therapies to be used on specific patient populations to ensure the greatest benefit.

Today, we know that gene expression analysis has enabled us to classify breast cancers according to their molecular diversity, and the five resulting molecular subtypes—luminal A, luminal B, HER2-enriched, basal-like and claudin-low—are not fully superimposable with the classical histopathological categories. As far as the triple-negative phenotype is concerned, the basal-like and claudin-low subtypes are most often represented in TNBC. More recently, cluster analysis of gene expression and ontology profiles has identified six TNBC subtypes, including two that were classed as basal-like [78]. These subtypes showed preferential responses to different therapeutic agents, for example basal-like subtypes (BL1 and BL2) showed higher expression of cell cycle and DNA damage response genes, and representative cell lines preferentially responded to cisplatin. On the other hand, mesenchymal and mesenchymal stem cell-like subtypes were enriched in gene expression for epithelial–mesenchymal transition, and growth factor pathways and cell models, and responded to BEZ235 (a PI3K/mTOR inhibitor) and the Abl/Src inhibitor dasatinib [78]. It is anticipated that advancements in the knowledge of molecular pathology of these subtypes will improve the design of more effective therapeutic strategies with targeted agents in TNBC.

Novel strategies have now reached advanced stages of clinical evaluation in TNBC patients. A summary of targeted agents in phase II or phase III clinical trials is reported in Table 1. These agents target angiogenesis (VEGF and VEGFR), DNA repair either directly with PARP inhibitors (e.g. olaparib, veliparib) or indirectly through DNA-binding (e.g. trabectedin) or damage potentiation (e.g. iniparib), and proliferation signalling (receptor TKIs and mTOR inhibitors). Unfortunately, we have to admit that clinical results have been somewhat disappointing so far, particularly in view of the enormous research efforts made worldwide in the last years. In fact, the only targeted agent currently available for patients with TNBC remains the anti-VEGF monoclonal antibody bevacizumab, which has not been specifically designed for TNBC.

The lack of systematic tumour tissue collection in most trials carried out in TNBC patients is of relevant concern for those who wish to delve deeper into this matter. It represents a major limitation for a translational approach to the huge body

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**Table 1. Summary of targeted agents in phase II or phase III clinical trials [79]**

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<tr>
<th>Target</th>
<th>Drug</th>
<th>Stage of development</th>
<th>Patient population</th>
<th>Regimen</th>
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<td>VEGFR</td>
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<td>Ramucirumab</td>
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<td>Sunitinib</td>
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<td>Sorafenib</td>
<td>Phase II</td>
<td>TNBC (neoadjuvant)</td>
<td>+ P/Cp</td>
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<tr>
<td></td>
<td>Apatinib</td>
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<td>mTNBC</td>
<td>Monotherapy</td>
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<tr>
<td></td>
<td>Cediranib</td>
<td>Phase II</td>
<td>Recurrent TNBC</td>
<td>+ Olaparib</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TNBC (neoadjuvant)</td>
<td>+ Ixabepilone</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mTNBC</td>
<td>+ Cp (BALI-1)</td>
<td>180</td>
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<td></td>
<td></td>
<td>mTNBC</td>
<td>+ CBp</td>
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<td>EGFR</td>
<td>Cetuximab</td>
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<td>TNBC (neoadjuvant)</td>
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<tr>
<td></td>
<td>Erlotinib</td>
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<td>mTNBC</td>
<td>+ Temsirolimus</td>
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</tr>
<tr>
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<td>Neratinib</td>
<td>Phase II</td>
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<td>Advanced/mTNBC</td>
<td>43</td>
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<tr>
<td>DNA</td>
<td>Lapatinib</td>
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<td>+ Everolimus</td>
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<td>PARP</td>
<td>Olaparib</td>
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<td>TNBC BRCA-mutated</td>
<td>Monotherapy</td>
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<td>PF-01367338</td>
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<td>Other</td>
<td>Iniparib</td>
<td>Phase III</td>
<td>mTNBC (stopped)</td>
<td>+ Gem/CBp</td>
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<td>+ P</td>
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<td>mTOR</td>
<td>Trabectedin</td>
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<td>Temsirolimus</td>
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<td>+ Neratinib</td>
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Anth, anthracycline; CBp, carboplatin; Cp, cisplatin; EGFR, epidermal growth factor receptor; Gem, gemcitabine; mTNBC, metastatic triple-negative breast cancer; mTOR, mammalian target of rapamycin; P, paclitaxel; PARP, poly(adenosine disphosphate-ribose) polymerase; T, docetaxel; Tax, taxane; TNBC, triple-negative breast cancer; VEGFR, vascular endothelial growth factor receptor.
of patient data produced by clinical trials up until now. We do believe that in the era of translational research in oncology, the utmost attention should be given to the issue of tissue collection in all trials involving a targeted agent in TNBC. In order to provide a truly targeted therapy, it is vital to identify subsets of patients who display predictive markers for response, and select these patients for treatment accordingly. Beginning from today, this is the only path we can pursue to limit our mistakes in designing and carrying out clinical trials, and to offer to our patients with TNBC the best quality clinical research.

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disclosures

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