Treatment of triple-negative metastatic breast cancer: toward individualized targeted treatments or chemosensitization?

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Triple-negative [estrogen receptor (ER)/-progesterone receptor (PR)/-HER2-] breast cancers account for ~15% of overall breast cancers. Triple-negative breast cancers demonstrate a panel of specific molecular alterations including a high rate of p53 mutations, frequent loss of function of BRCA1, phosphatase and tensin homolog (PTEN) loss and a specific panel of tyrosine kinase activation [fibroblast growth factor receptor 2 (FGFR2)]. This molecular entity is considered as sensitive to chemotherapy in the adjuvant setting. When metastatic, the disease is usually aggressive and drug resistant, leading to cancer death within 18 months for the majority of patients. There is no evidence from randomized trials that triple-negative breast cancers have a different sensitivity to specific chemotherapy compared with other molecular classes. Similar findings have been reported for bevacizumab. Several recent research efforts have focused on this entity in the last few years. DNA alkylating agents have shown promising activity in the neoadjuvant setting, but no evidence from a phase III trial currently supports its use. Several targeted therapies are also being successfully developed. Poly(ADP ribose) polymerase 1 (PARP1) inhibitors induce tumor response as a single agent in BRCA1-mutated breast cancer, and could sensitize cisplatin in the whole triple negative population. Several other targeted agents are being developed in this setting, including epidermal growth factor receptor (EGFR), FGFR2, mammalian target of rapamycin (mTOR) and NOTCH inhibitors.

**Key words:** alkylating agents, molecular alterations, targeted agents, triple-negative breast cancers

**definition and characteristics of triple-negative breast cancers**

Breast cancer is the leading cancer among women. Improved screening, imaging and treatment have improved survival. The 10-year overall survival rate for unselected breast cancers now ranges between 70% and 85% in different countries [1]. High throughput technologies have shown that breast cancer includes several molecular entities, i.e. luminal A, luminal B, HER2-overexpressing and basal-like breast cancers [2–4]. Basal-like breast cancers account for 15% of overall breast cancers. For ease, basal-like breast cancers have been referred as triple-negative breast cancer (TNBC), i.e. those that do not overexpress estrogen receptor (ER), progesterone receptor (PR) or HER2. Nevertheless, several reports suggest that TNBC does not totally overlap with the basal-like phenotype. Nielsen et al. [5] have defined basal-like breast cancer as ER−/HER2− cytookeratin 5-6 (CK5-6)+ and/or epidermal growth factor receptor (EGFR)+ breast cancers. Based on this definition, Biddard et al. [6] have shown that TNBCs include both basal-like breast cancer and a subset of poorly differentiated, highly proliferative luminal breast cancer. It must be emphasized that this topic is a matter of controversy as Kreike et al. have reported a perfect overlap between the basal-like and triple-negative phenotype [7]. Overall, the TNBC population is at least represented in patients who have a basal-like phenotype.

TNBCs demonstrate some specific clinicopathological characteristics. They are associated with a younger age, a high mitotic index and a poor prognosis [8]. Although TNBC is sometimes referred to as a refractory disease, recent biomarker studies have clearly shown that adjuvant chemotherapy is associated with a high efficacy. Rouzier et al. [9] have shown that neoadjuvant chemotherapy was associated with a high likelihood of a pathologically documented complete response (pathological complete response) in this subset of patients, which correlates with better outcome. Also, in the study reported by Conforti et al. [10], adjuvant chemotherapy was associated with an adjusted hazard ratio for relapse or death of 0.54 [95% confidence interval (CI) 0.27–1.08] in patients with a basal-like phenotype. Unfortunately, patients who are refractory to chemotherapy will relapse early, with a peak of metastases occurring at 1 year [11]. For those patients who undergo a metastatic relapse, life expectancy is very poor. In a study that included 284 patients [11], the median survival after metastatic relapse was 1 year in TNBC compared with 2.3 years for the other subtypes.
Overall, TNBC appears to be an aggressive disease for which a subset of patients with early stage breast cancers are cured by chemotherapy. The remaining patients usually experience early metastatic relapse with very poor outcome.

**conventional treatments of metastatic TNBC: do they exhibit some specific sensitivity?**

No targeted agent that interacts with the oncogenic pathway has yet been registered in patients with TNBC. In all of the recent randomized trials performed in the first-line metastatic setting, no evidence of a specific sensitivity of TNBC has been observed. As an illustration in chemotherapy trials, O’Shaughnessy et al. [12] reported that adding capecitabine to docetaxel provided benefit whatever the ER status. Similar observations were made in the trials that evaluated the combination gemcitabine/vinorelbine [13] and gemcitabine/paclitaxel [14]. In a recent analysis of five phase II studies and two phase III trials, Perez et al. found that the addition of ixabepilone to capecitabine results in an increase in median progression-free survival (PFS) for patients with TNBC versus capecitabine alone; comparable with that seen in patients with non-triple-negative tumors [15]. Finally, in randomized trials that evaluated the efficacy of bevacizumab, no difference in the level of efficacy was observed between triple-negative and other subtypes [16, 17]. Overall, these data suggest that, when adjusted for previous drug exposure, TNBCs show the same level of relative efficacy (hazard ratio for response and progression) for conventional treatment. Nevertheless, a specific characteristic of TNBC relates to its presentation at the time of metastases. As previously mentioned, although a minority of patients with TNBC present with stage IV, most of the patients with metastases have relapsed shortly after (neo) adjuvant chemotherapy. These patients should therefore be considered as resistant to taxanes and anthracyclines. Another specific characteristic of TNBC relates to the site of metastasis. In the study by Liedkte et al. [11], 74% of the patients relapse in the viscera (liver and lung). They also develop brain metastases earlier in the course of disease [18].

Overall, while having the same level of sensitivity to other drugs, TNBCs are a more aggressive disease that are usually drug resistant due to previous adjuvant treatment. In these circumstances, what could be the optimal treatment in a patient with TNBC relapsing in the viscera 1 year after anthracycline-/taxane-containing chemotherapy? Although recommendations suggest that sequential chemotherapy is an appropriate option in metastatic breast cancers, the same guidelines acknowledge that combination therapy is recommended in the case of aggressive disease [19]. Taking the hypothesis that a patient is resistant to taxanes, the combination of capecitabine and vinorelbine, or gemcitabine and vinorelbine is acceptable. Also, some new drugs have shown efficacy in this setting of taxane resistance. Nab-paclitaxel, an albumin-bound paclitaxel, has shown a 13% response rate in a population of 15 patients pre-treated with paclitaxel [20]. Also, ixabepilone, in combination with capecitabine, has shown efficacy in patients with anthracycline-/taxane-resistant disease. In a trial that included 752 patients, ixabepilone was associated with a 25% decrease in the risk of disease progression (hazard ratio 0.75; 95% CI 0.64–0.88) [21]. This latter drug is not approved in Europe. Finally, bevacizumab has shown a significant reduction in the hazard of progression in four randomized trials [16, 17, 22, 23]. The interesting findings regarding bevacizumab trials are the consistent increase in the response rates provided by chemotherapy alone. As an illustration, in the E2100 trial [16], adding bevacizumab to paclitaxel increased response rates from 21.2% to 36.9% in the overall population (P <0.001). This latter feature can be a decision criterion in patients for whom a tumor regression is needed, and who are chemorefractory.

Overall, this section emphasizes the fact that, given the aggressiveness and resistance of TNBC, all the options proposed in first-line treatment are associated with a failure within a few months. Novel strategies are therefore needed.

**DNA alkylating agents: a TNBC-specific drug family?**

TNBCs are characterized by a deficiency in the DNA repair machinery. Based on this background, it has been hypothesized that DNA alkylating agents could be specifically effective in this subset of patients. Only a few clinical data, if any, actually support this hypothesis. In a recent publication, Silver et al. [24] reported a 22% rate of pathological complete response to neoadjuvant chemotherapy, in a study of 28 TNBCs. In the context of metastatic breast cancer, O’Shaughnessy et al. [25] have reported a median PFS at 3 months in patients treated with carboplatin/gemcitabine. Several data actually suggest that, within the population with TNBC, those exhibiting a BRCA1 mutation could be highly sensitive to cisplatin [26]. Several other alkylating agents have been evaluated in small series. Finally, trabectedin was recently reported to have efficacy in TNBC [27].

Overall, these data suggest that it is too early to reach a conclusion on the efficacy of cisplatin and trabectedin in patients with TNBC. Nevertheless, some preliminary data suggest that these drugs could be specifically effective in patients with BRCA1 germline mutations, highlighting a potential split in tumor biology between TNBC/BRCA– and TNBC/BRCA+ breast cancers.

Although these data could be considered as encouraging, they emphasize the need for new therapies that would specifically interact with oncogenesis pathways.

**biology of TNBC: in search of candidate targets**

TNBC arises from myoepithelial cells and therefore shares with these cells their surface biomarkers including CK5-6 and EGFR [28]. In recent years, the molecular characteristics of TNBC have started to be deciphered. Major targets under investigation are reported in Figure 1. Basal-like breast cancer is characterized by a defect in p53 and the DNA repair pathway, leading to genetic instability [29]. DNA repair defects usually relate to lack of BRCA1 function. This loss of function can be due to either germline mutations or underexpression. Several
explanations have been put forward to explain the underexpression of BRCA1, e.g. promoter methylation [30] or ID4 overexpression [31]. Overall, it is considered that a majority of basal-like breast cancers have a defect in BRCA1 function. Interestingly, when genomic profiling of TNBC is compared with that of benign lesions, the DNA repair pathway is one of the most often overexpressed pathways [32]. This overexpression could be interpreted as a compensatory mechanism of the DNA repair defect. Among the genes which are overexpressed, those for checkpoint kinase 1 (CHEK1) and poly(ADP ribose) polymerase (PARP1) emerge as candidate targets that could be inhibited. When looking at dysregulation of kinases, some specific alterations are observed in TNBC. Phosphatase and tensin homolog (PTEN) is a protein that inhibits activation of the AKT/mammalian target of rapamycin (mTOR) pathway. PTEN losses have been observed in up to 30% of TNBCs [33]. Interestingly, such PTEN losses have been associated with activation of AKT in TNBC samples [34]. These data suggest that there is a rationale to evaluate mTOR inhibitors in patients with TNBC. EGFR overexpression is a hallmark of TNBC; it is present in ~45–70% of TNBCs [35]. Nevertheless, only a few data support its activation in patients with TNBC. Interestingly, fibroblast growth factor receptor 2 (FGFR2) has recently been reported to be amplified in a subset of patients with TNBC [31]. In the same paper, the authors showed that FGFR2 inhibition led to a decrease in cell proliferation, in cell lines where amplification was observed.

TNBC has also been reported to have specific alterations in host–cancer relationships. In a comparative genomic hybridization (CGH) array study, vascular endothelial growth factor A (VEGFA) was reported to be increased in 34% of TNBCs [33]. Such a gain correlated with overexpression, a finding that could explain the high rate of TNBCs with positive immunostaining for VEGF. TNBC was also reported as having a discrete pattern of chemokine release, together with a high level of T cell infiltration [36]. Interestingly, several reports have suggested that such immune activation could be associated with a good prognosis [37].

It has been shown recently that the androgen receptor is overexpressed in a subset of patients with TNBC [38]. This steroid receptor mediates gene transcription and cell proliferation. A role for this receptor has been shown in prostate oncogenesis.

Finally, basal-like breast cancers have been reported to be enriched in breast cancer stem cells (CD44+/CD24−/low) [39]. Such cells have a dysregulation in the NOTCH pathway. Interestingly, CGH profiling of basal-like breast cancer has suggested that this subset of cancer could specifically demonstrate NOTCH4 amplification [31].

Overall, these data suggest that several candidate targets have been reported in recent years. Below, we will review the clinical implications of such molecular findings.

**targeting the DNA repair pathway in TNBC**

PARP1 is an enzyme involved in nucleotide excision repair (NER). Two therapeutic strategies can be followed when targeting PARP1. First, PARP1 inhibitors could be given as a single agent in BRCA1-mutated TNBC. In this approach, PARP1 inhibitors would lead to synthetic lethality by hitting a second DNA repair pathway (NER). Several compounds have already been evaluated in phase II trials. Tutt et al. [40] have reported efficacy data for olaparib in 27 patients with BRCA1/2 mutations. Forty-five percent response rates were reported in this heavily pretreated population. Another strategy aim is to inhibit PARP1 in order to sensitize cancer cells to DNA alkylating agents. In this strategy, PARP1 is considered as a player involved in the resistance to cisplatin. In a phase II randomized trial that included 116 patients with TNBC, O’Shaughnessy et al. [25] reported that treatment with PARP1 inhibitors was associated with a hazard of progression of 0.34 (95% CI 0.200–0.584).

Overall, these two studies emphasize that PARP1 is a major target in TNBC. Several studies are ongoing to validate such findings. Several mechanisms of resistance to PARP1 inhibitors have been described. These data emphasize the need for treatments that could reverse resistance to PARP1 inhibitors. As previously stated, CHEK1 is a gene overexpressed in TNBC. This protein is involved in the cell cycle checkpoint, and mediates a halt of the cell cycle in the case in genomic alterations. CHEK1 inhibition has been reported to mediate mitotic catastrophe and death in cancer cells. Several new compounds are being developed in order to target CHEK1 in TNBC.

**kinase inhibitors for TNBC**

As previously stated, several kinases are specifically activated in TNBC. EGFR inhibitors have been evaluated in several studies. In a phase II randomized trial performed in TNBC, Carey et al. [41] reported that adding cetuximab to carboplatin did not improve outcome. In this study, the response rates were 17% in the two arms. Interestingly, cetuximab alone was associated with a 6% response rate, suggesting that this drug could provide some efficacy in selected patients.

Recently Harbeck et al. have reported that BIBW 2992, a novel oral, irreversible EGFR and HER2 inhibitor, achieved clinical benefit in 13% of the 29 metastatic TNBC patients [42]. These data suggest that EGFR inhibition is not associated with a major improvement in the outcome of TNBC.
Nevertheless, it must be pointed out that the EGFR gene is amplified in a very few number of cases, and that optimal evaluation of such targeted agents should stratify clinical trials on EGFR amplification status. The occurrence of objective responses with an EGFR inhibitor alone suggests that a small subset of patients could derive some benefit. Also, the failure of EGFR inhibitors could be related to activation of alternative pathway. As an illustration, TNBC presents a high rate of PTEN loss and AKT activation [33]. One could argue that EGFR inhibitor would not be effective in TNBC which showed PTEN loss in addition to EGFR activation. This consideration would suggest that EGFR inhibitors should be developed as combination therapy with mTOR inhibitors.

FGFR inhibitors represent a new drug family. At least four compounds are currently under clinical trials. These drugs are either FGFR specific or target FGFR as part of their tyrosine kinase panel, in addition to VEGFR inhibition. Several trials (cTK12202) include patients with the triple-negative phenotype. Nevertheless, given the very low frequency of the amplification, it is unlikely that some patients with FGFR2-amplified breast cancer will be included in such clinical trials. Dedicated trials would be welcome to address this issue.

mTOR inhibitors have been shown to improve outcome in several cancer types, including renal cancer. As previously reported, TNBC presents a high frequency of PTEN loss and mTOR activation. There is therefore a rationale to develop mTOR inhibitor in patients with TNBC that show PTEN loss. Interestingly, several papers also report that mTOR activation could lead to cisplatin resistance, a phenomenon reversible by everolimus [43]. Iwan et al. [43] reported that adding everolimus to cisplatin could increase by 5-fold the loss of viability in vitro. These data suggest that there is a rationale to combine cisplatin and mTOR inhibitors in patients with TNBC.

targeting the host

Cancer is infiltration by many cells from the host that contribute to tumor development. Angiogenesis is regulated by VEGFA. Interestingly, a subset of TNBC shows an increase in the VEGFA gene [33]. Unfortunately, until now, there has been no evidence that TNBC could be highly sensitive to bevacizumab. Nevertheless, in a phase II trial that evaluated sunitinib single agent, this drug was associated with a 15% response rate in patients with TNBC [44]. Although the sample size was small, the observation of an objective response in a few patients once again suggests that the drug could have some effect in selected patients. Further studies should be driven by biomarkers and address the sensitivity of VEGFA-amplified TNBC to the single agent sunitinib. Once again VEGFA inhibitors could also be given with the aim of sensitizing chemotherapy. As previously reported, VEGFA inhibitors significantly improve response rates observed with conventional cytotoxics [16, 17]. Efforts are being made to understand and improve this synergism. Fine-tuning the administration schedule of VEGF inhibitors and incorporating vascular disrupting agents could further increase such synergism.

TNBC is characterized by infiltration of immune cells and expression of interferon-related genes [36]. The recent development of new immune adjuvants [ligands for Toll-like receptors (TLRs)], together with the discovery of new antigens (WT1, NYESO, etc.) suggests that there is room for the development of cancer vaccines in TNBC. Nevertheless, as pointed out earlier, metastatic TNBC usually present as a very aggressive disease, a characteristic that is not compatible with optimal vaccination. These considerations suggest that cancer vaccines would be better placed in the adjuvant setting.

targeting the stem cells

Stem cells are self-renewing cells that show resistance to conventional treatment and allow repopulation of the cancer. These cells possess some specific molecular alterations including activation of the Hedgehog and NOTCH pathway. In CD34+ leukemic cells, drug resistance is restored by Hedgehog inhibitors [45]. Several targeted agents under development specifically target this pathway. GDC-0449, a Hedgehog antagonist, is currently being tested in association with a γ-secretase inhibitor (RO4929097) in advanced breast cancer [46].

implications in daily practice

As described above, two different strategies are being developed in the field of TNBC (see Table 1). In the first approach, the drug is considered as a targeted agent that inhibits a specific molecular alteration. In this approach, each drug family is being developed in biologically defined populations. As illustrations, olaparib was developed in patients with BRCA1 mutations, which account for a limited number of TNBC

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Table 1. Single drug driven by biology or chemosensitizers to treat metastatic TNBC?

EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; mTOR, mammalian target of rapamycin; PARP1, poly(ADP ribose) polymerase; PTEN, phosphatase and tensin homolog; TNBC, triple-negative breast cancer; VEGFA, vascular endothelial growth factor A.
patients [40]. Using the same approach, FGFR inhibitors could be developed in patients with FGFR2 amplification. This approach will require the development of a bioassay for patient selection. With this approach, the biomarker is being developed as a companion to the drug. In the second approach, the drug could be given as a chemosensitizer to ‘all comers’ with TNBC. This approach was used for the development of BSI-201 in TNBC [25]. The same approach could be used for everolimus since this drug has been shown to reverse resistance to cisplatin. Similarly, treatment targeting the host and the stem cells could belong to the same strategy. In this approach, the predictive biomarker (if any) is identified after clinical trials, and requires a large number of patients. Since all approaches are being run in parallel, it is very likely that some drugs will emerge in the molecular subset of TNBC (FGFR) while others will be developed in all comers (BSI-201). Several specific problems are being encountered with both strategies. In the first scenario, the emerging problem has been named ‘orphan molecular disease’. Indeed, all the previously discussed alterations including loss of PTEN, FGFR2 amplification and EGFR amplification, but also AKT mutation, etc. are seen overall in only a few patients, creating difficulties in performing randomized studies and the development of dedicated bioassays. For these patients, there is a need to develop molecular screening using high-throughput technologies, and to allow drug registration based on small biogy-driven phase II trials.

In the second scenario, i.e. when the targeted agent is being used to sensitize chemotherapy, there is a need to keep chemotherapy as the cornerstone of treatment, and there is the risk of adding molecular alterations to a disease that could have been sensitive to targeted agents.

disclosure

Authors have nothing to disclose.

references


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