Pathol. Oncol. Res. (2018) 24:701–716
DOI 10.1007/s12253-017-0307-2

REVIEW PAPER

Therapeutic Implications of the Molecular and Immune Landscape of Triple-Negative Breast Cancer

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Received: 2 July 2017 / Accepted: 4 September 2017 / Published online: 14 September 2017

Abstract Treatment and management of breast cancer imposes a heavy burden on public health care, and incidence rates continue to increase. Breast cancer is the most common female neoplasia and primary cause of death among women worldwide. The recognition of breast cancer as a complex and heterogeneous disease, comprising different molecular entities, was a landmark in our understanding of this malignancy. Valuing the impact of the molecular characteristics on tumor behavior enabled a better assessment of a patient's prognosis and increased the predictive power to therapeutic response and clinical outcome. Molecular heterogeneity is also prominent in the triple-negative breast cancer subtype, and is reflected by the distinct prognostic and patient's sensitivity to treatment, being chemotherapy the only systemic treatment currently available. From a therapeutic perspective, gene expression profiling of triple-negative tumors has notably contributed to the exploration of new druggable targets and brought to light the need to align these patients to the various therapies according to their triple-negative subtype. Additionally, the higher amount of tumor infiltrating lymphocytes, and the prevalence of an increased expression of PD-1 receptor and its ligand, PD-L1, in triple-negative tumors, created a new treatment opportunity with immune checkpoint inhibitors. This manuscript addresses the current knowledge on the molecular and immune profiles of breast cancer, and its impact on the development of targeted therapies, with a particular emphasis on the triple-negative subtype.

Keywords Breast cancer · Triple-negative breast cancer · Intrinsic subtype · Gene expression profiling · Targeted therapies · Immune checkpoint inhibitors

Introduction

Human breast tumors differ in their natural history and responsiveness to therapy [1]. Currently, disease management relies on well-validated clinico-pathological prognostic variables that include tumor size, lymph node status, proliferation index and tumor histological characteristics [2–7]. Prognostic signatures determined by the expression of estrogen (ER) and progesterone (PR) receptors, and the human epidermal growth factor receptor 2 (HER2), further aid in the stratification of patients, and are regarded as the main drivers for the selection of suitable therapeutic options [8–11]. Nonetheless, the systematic analysis of gene expression patterns in human breast tumors, contributed to the current knowledge of breast cancer molecular complexity and identified distinctive molecular portraits that unveiled similarities and differences among the tumors [12].

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Molecular features of breast tumors

Through hierarchical clustering analysis of gene expression profiling, Perou *et al*. identified four biologically distinct disease entities – luminal, HER2-enriched, basal-like and normal breast-like [12]. The distinction between two luminal-like subtypes – luminal A and luminal B – was further uncovered by Sørlie *et al*., which were not evident with the traditional histopathological methods [13, 14]. The expression of ER and ER-related genes, proliferation-related genes, and HER2 and other genes mapping to the region of HER2 amplicon on chromosome 17 were the major drivers determining the molecular subtypes [12–15]. Subsequent studies additionally identified a claudin-low intrinsic breast cancer subtype characterized by the low expression of genes involved in tight junctions and cell-cell adhesion, including *Claudins 3*, *4*, *7*, *Occludin*, and *E-cadherin*, and enriched in immune response genes and stem cell-associated features [16, 17]. In fact, these tumors displayed a phenotype that closely resembled the epithelial stem cell in the normal mammary epithelial differentiation hierarchy [17]. A recent study additionally revealed the relatively high incidence of ER-positive tumors (36%) and non-triple-negative tumors (48%) within the claudin-low subtype, compared to the luminal A (95%) and basal-like (24%) subtypes, respectively, suggesting that the claudin-low subtype is much more heterogeneous than the other two subtypes [18]. The recognition of intrinsic biological subtypes within the breast cancer spectrum has now become clinical practice through the use of the common immunohistochemical approach [19]. As illustrated in Fig. 1, the combined evaluation of ER, PR, HER2 protein overexpression and/or oncogene amplification, and Ki-67 labeling index was adopted for a simplified classification of breast tumor subtypes [19–21]. Among the breast cancer intrinsic subtypes, the basal-like group has generated much interest due to its substantial overlap with a subset of tumors with a triple-negative immunohistochemical signature.

The immunohistochemical analysis of ER, PR, HER2 and Ki-67 enabled to establish a breast cancer classification reflecting the intrinsic subtypes. An empiric cutoff of ≥20% PR-positive tumor cells was statistically chosen and proved significant for predicting survival differences within immunohistochemical-defined luminal A tumors [22]. In luminal B breast tumors, standardized cutoffs for Ki-67 have not been established and might vary between laboratories. Nonetheless, the 20% threshold was accepted as indicative of high Ki-67 status [23], although others have proposed a cutoff of 14% [20]. The triple-negative tumors do not overlap completely with the basal-like subtype [24–26], and also includes some special histological types such as medullary and adenoid cystic carcinoma with low risks of distant recurrence [27].

Nevertheless, it is recognized that molecularly- and immunohistochemically-defined classes do not overlap completely [25, 26, 24]. Despite this topic still being subject for some controversy, other panels of immunohistochemical surrogate markers, which include the expression of cytokeratins 5/6 and/or EGFR, have been proposed to define basal-like breast cancers [28, 25, 24]. According to this classification, approximately 74–84% of triple-negative breast cancers (TNBC) express basal cytokeratins and EGFR [24, 25]. Therefore, it is reasonable to assume that the triple-negative identity comprises two subsets of breast tumors characterized by basal- and non-basal-like phenotypes [26].

Current therapeutic approaches

In the setting of early breast cancer, surgery (mastectomy or breast-conserving surgery) and radiotherapy play an important role in the management of the disease [29, 30]. However, the value of surgery in patients with advanced breast cancer is still under debate [31–34], and systemic therapy is the predominant treatment in this setting [35]. Fig. 2 briefly captures the class of systemic treatment as a function of breast cancer subtype, while Table 1 summarizes the therapeutic agents being currently used for the treatment of breast cancer.

The clinico-pathological surrogate definitions resembling intrinsic subtypes of breast cancer guide selection of systemic adjuvant therapies.

Endocrine therapy

Endocrine therapy is the preferred option for hormone receptor-positive disease, even in the presence of visceral metastases (Table 1) [35]. It is characterized by a significant activity in the treatment of patients with luminal A disease [42, 43], being the expression of Ki-67 a biomarker for survival [44, 45]. Tamoxifen is the standard of care for premenopausal women. The value of suppressing ovarian function has been a topic of controversy, particularly in patients previously treated with chemotherapy [29, 46, 47]. Arguments favoring the inclusion of ovarian suppression have been recently addressed in international consensus guidelines, and included age of 35 or less, the persistence of premenopausal estrogen level after adjuvant chemotherapy, or the involvement of four or more axillary nodes [48, 30, 35]. In patients contraindicated to Tamoxifen, a luteinizing hormone-releasing hormone (LHRH) agonist, in combination with an aromatase inhibitor, is indicated (Table 1) [29, 35, 30].

The luminal B subtype has relatively lower benefit from endocrine treatment, partially due to a low expression of estrogen receptors [49, 10], being inherently more aggressive than the luminal A. It benefits from a more aggressive therapy and is generally treated with both endocrine therapy and

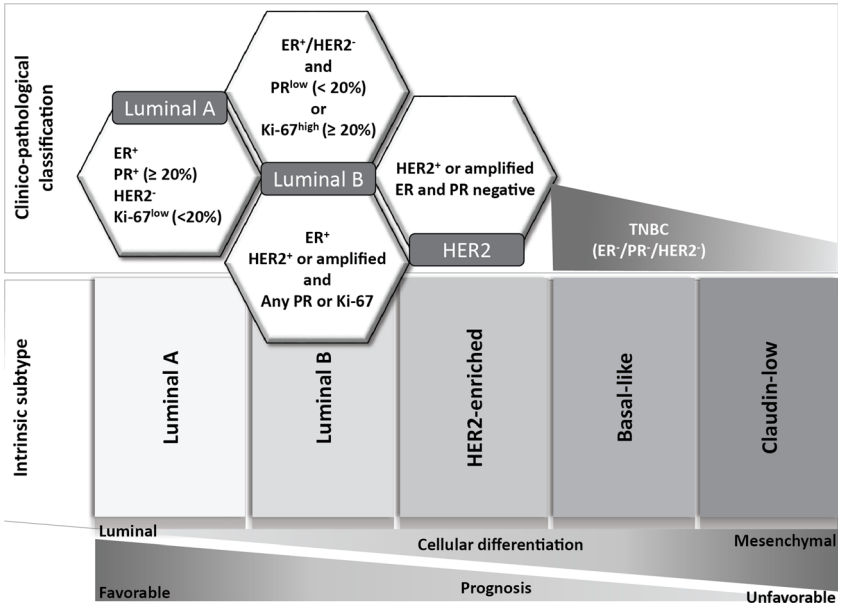

Fig. 1 Surrogate definitions of intrinsic subtypes of breast cancer

cytotoxic agents (Table 1) [50, 51, 29, 30, 35]. Although luminal A disease is usually less responsive to chemotherapy [50, 51], a few selected patients at higher risk of relapse (extensive nodal involvement) might also benefit from it [30].

Anti-HER2 therapy

The overexpression of HER2 in luminal tumors have also been associated with increased relapse rate in patients treated with endocrine therapy, compared with HER2-negative tumors [52]. In the former, the combination of endocrine and anti-HER2 therapy revealed a significant therapeutic benefit (Table 1) [53, 54, 35, 30, 29]. Recent evidence further suggested that complete resistance to both Anastrazole and Trastuzumab sequential monotherapies, can be overcome in a proportion of patients upon their simultaneous administration [55].

Trastuzumab is a keystone systemic therapy for (non-luminal) HER2-overexpressing tumors [56]. The combination of this monoclonal antibody with chemotherapy has improved overall survival and reduced the risk of disease recurrence in the adjuvant setting (Table 1) [57–59]. However, increased cardiac dysfunction has been observed when Trastuzumab is associated with anthracycline-based chemotherapy [60, 59]. Notwithstanding, Slamon *et al*. demonstrated that similar disease-free or overall survival could be attained with a taxane-based regimen, together with a lower risk of cardiotoxicity [59].

Chemotherapy

Among breast cancer subtypes, the triple-negative constitutes one of the most challenging groups and where chemotherapy

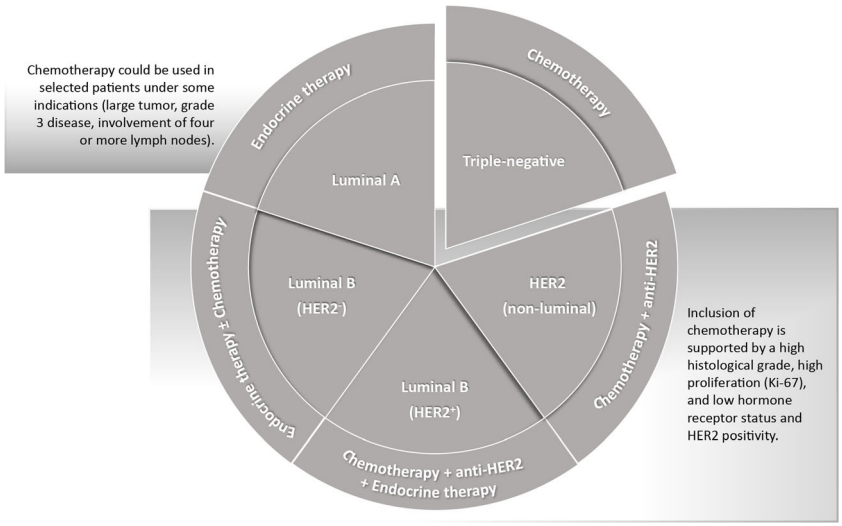

Fig. 2 Systemic treatments recommended for different breast cancer subtypes

Table 1 Therapeutic agents currently used in the treatment of breast cancer

Type of therapy	Drug	Class	Target
Endocrine [35–37]	Tamoxifen	Estrogen receptor modulator	Estrogen receptor
	Anastrozole Letrozole	Aromatase inhibitor	Aromatase enzyme
	Exemestane		
	Fulvestrant	Estrogen receptor downregulator	Estrogen receptor
	Goserelin	LHRH blocker	LHRH receptor
Anti-HER2 [35, 38, 39]	Trastuzumab	Monoclonal antibody	HER2 receptor
	Lapatinib	Small molecule inhibitor	HER2/EGFR tyrosine kinase pathways
	Pertuzumab	Monoclonal antibody	HER2 receptor
	Trastuzumab-emtansine	Antibody-cytotoxic agent	HER2 receptor/tubuline
Chemotherapy [35, 40]	Doxorubicin Epirubicin	Anthracyclines	
	Liposomal doxorubicin		
	Paclitaxel Docetaxel	Taxanes	
	Nanoparticle albumin-bound paclitaxel		
	Gemcitabine Capecitabine	Antimetabolites	
	Fluorouracil		
	Vinorelbine	Vinca alkaloids	
	Ixabepilone	Epothilone B analog	
	Carboplatin Cisplatin	Platinum agents	
	Cyclophosphamide	Alkalyting agent	
Skeletal metastases-targeted therapy [35, 41]	Zelodronic acid Clodronate	Bisphosphonates	Osteoclast function
	Denosumab	Monoclonal antibody	RAKNL

plays a crucial role. The interest in TNBC arises from the current absence of targeted therapies for this group of patients, associated with a poor prognosis. At present, the only systemic therapy available for patients with triple-negative breast disease is chemotherapy (Table 1). Current treatment strategies include anthracyclines, taxanes, ixabepilone and platinum agents. Interestingly, triple-negative breast tumors are more sensitive to chemotherapy than the other subtypes, an observation supported by a number of studies on neoadjuvant chemotherapy [61–63]. The strong association of triple-negative breast tumors with germline mutations in the *BRCA1* gene [64] has also attracted attention to the potential use of platinum-based compounds in TNBC therapy (Table 1) [65–68]. However, platinum agents failed to demonstrate improved benefit in the context of advanced breast cancer [69], and warrant further elucidation on their efficacy. Platinum-based chemotherapy is currently recommended only for patients with known *BRCA* mutation [35, 30]. In patients with breast cancer bone metastases, the routine use of bone-modifying agents, such as bisphosphonates or denosumab (Table 1), are advised in combination with other systemic therapy [35].

Molecular landscape of triple-negative breast tumors

Triple-negative breast cancer represents a major hurdle in breast disease, due to the absence of well-defined molecular targets. Alike the previously described intrinsic subtyping of breast cancer, the molecular dissection of triple-negative tumors has also refined our knowledge about the biology underpinning this disease. Furthermore, profiling of TNBC subtype facilitated the identification of targetable vulnerabilities within different subsets, and contributed to the development of targeted therapeutic strategies and identification of biomarkers for efficacy to standard chemotherapy. Recent studies of gene expression profiling have uncovered the heterogeneous nature of TNBC. Lehmann *et al*. identified seven TNBC subtypes

characterized on the basis of gene ontologies and differential gene expression patterns [70]. These subclasses were named as basal-like (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), luminal androgen receptor (LAR), and unstable subtypes.

Both BL1 and BL2 tumors are enriched for genes associated with proliferation and DNA damage response. The highly proliferative nature of BL1 tumors was further supported by an elevated Ki-67 assessed both by mRNA expression and immunohistochemical staining analysis. Additionally, the BL2 subtype displayed a gene signature characterized by growth factor and metabolic signaling, and myoepithelial markers [70].

Tumors in the immunomodulatory subclass overexpress genes involved in immune and cytokine signal transduction pathways, including T-cell associated genes, interferon regulatory factors and tumor necrosis factor [70]. Interestingly, the immunomodulatory gene signature largely overlapped with the one of medullary breast cancer [71], a rare pathological type of cancer associated with a better prognosis, despite the presence of aggressive features, such as large tumor size and a high nuclear grade [72].

Both the mesenchymal and MSL subtypes shared a high expression of genes involved in the epithelial-mesenchymal transition and growth factor signaling pathways, such as mTOR, Wnt/β-catenin, and TGF-β. Additionally, MSL tumors are distinguished by a unique gene signature involving growth factor signaling pathways (FGFR, VEGF and PDGFR), decreased expression of proliferation-related genes and enrichment in pluripotency-related genes [70]. The rare and histologically diversity of metaplastic breast cancer, characterized by a propensity for distant metastases and resistance to standard chemotherapy, shared similar features with these two molecular defined subtypes [70, 73].

Finally, the LAR group constituted a luminal subtype driven by the androgen receptor signaling and hormone-regulated pathways [70]. Androgen signaling had been previously reported in ER-negative tumors, coupled with a molecular apocrine gene expression signature and associated to tumors with strong histological apocrine features [74].

Noteworthy, subtype-specific pathologic complete responses (pCR) were reported by Masuda *et al*. in a retrospective analysis performed in biopsies from patients treated with neoadjuvant chemotherapy. Patients with the BL1 tumors achieved the highest pCR rate (52%) in contrast to patients with BL2 and LAR tumors, who showed the lowest response rates (0% and 10 %, respectively) [75]. More recently, other groups confirmed the existence of distinct TNBC molecular profiles and also associated them to different prognoses [76, 77].

The Cancer Genome Atlas Network further analyzed the genomic heterogeneity of breast cancers by integrating information across different platforms. *TP53* mutation or deletion was the most common aberration identified in TNBC, being observed in 80% of the cases, followed by genomic aberrations in the PI3K pathway [78]. Abramson *et al*. extended this analysis and validated these findings [79], thus suggesting that specific subsets of TNBC displaying increased PI3K pathway activity might benefit from treatment with PI3K inhibitors [80, 81].

The high frequency of p53 dysfunction in TNBCs likely results from defects in the DNA repair pathway, and is consistent with the significant genomic instability that characterizes these tumors. This feature is also common to tumors carrying mutations in the *BRCA1* gene [82]. Although *BRCA1* mutations in sporadic basal-like breast cancers are rare [83], they display a *BRCAness* immunophenotype, resulting from the impairment of double-strand break repair through homologous recombination [82]. Such dysfunction has important clinical relevance because double-strand break impairment is the basis for targeted treatments [84].

Altogether, these results bear important implications in the way triple-negative breast cancer is managed. Gene expression profiling analysis of TNBCs greatly contributed to the exploration of new druggable targets for the treatment of this disease. Furthermore, acknowledging the heterogeneity of this group uncovered the need to align patients to the various therapies according to their triple-negative subtype.

Targeted therapies for triple-negative breast cancer

PARP inhibitors

Poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors (Table 2) have raised great interest in the setting of TNBC due to the *BRCAness* phenotype of these tumors. These inhibitors have shown promising results in *BRCA1/2*-mutant tumors, including in breast cancer [85]. However, clinical studies with PARP inhibitors, olaparib [86, 87] or veliparib [88], failed to demonstrate a significant response in patients with TNBC. Additionally, the combination of iniparib with chemotherapy, which had previously shown to improve survival of patients with metastatic TNBC in a phase II clinical study [89], was not confirmed in the phase III trial [90]. Overall, PARP inhibitors demonstrated limited benefit in *BRCA* unselected TNBC populations, either as a single regimen [87] or in combination with other chemotherapeutics [88, 91]. A recent study demonstrated that an increased level of allelic imbalance copy number aberrations and expression of meiosis-associated gene *HORMAD1* in triple-negative tumors correlated with higher sensitivity to platinum salts and PARP inhibitors [92]. These results clearly underlie the need to identify the subset of patients with triple-negative disease that may benefit from treatment with PARP inhibitors.

Table 2 Clinical trials of most relevant targeted therapies for the treatment of triple-negative breast cancer

Targeted therapy	Therapeutic combinations	Clinical phase	ClinicalTrial.gov Identifier (Ref.)
PARP inhibitors			
Olaparib (AZD2281)	Monotherapy	II	NCT00494234 ([87])
			NCT00679783 ([86])
	Cediranib Maleate	I/II	NCT01116648
	Paclitaxel	I	NCT00707707 ([91])
Velipalib (ABT888)	Temozolomide	II	NCT01009788 ([88])
Iniparib (BSI-201)	Gemcitabine/Carboplatin	III	NCT00938652 ([90])
	Irinotecan	II	NCT01173497
mTOR inhibitors			
Everolimus (RAD001)	Paclitaxel followed by 5-Fluorouracil/Epirubicin/	II	NCT00499603 ([94])
	Cyclophosphamide		
	Cisplatin and Paclitaxel	II	NCT00930930 ([93])
	Carboplatin	II	NCT01127763 ([95])
			NCT02531932
Temsirolimus	Erlotinib	I	NCT00998036
	Neratinib	I/II	NCT01111825
	Liposomal doxorubicin/ Bevacizumab	II	NCT02456857
PI3K inhibitors			
BMK120	Capecitabine	II	NCT02000882
GDC-0941	Cisplatin	I/II	NCT01918306
AZD8186	Monotherapy	I	NCT01884285
Akt inhibitors			
Ipatasertib	Paclitaxel	II	NCT02162719 ([102])
			NCT02301988 ([101])
GSK2141795	Trametinib	II	NCT01964924
Androgen receptor/synthesis inhibitors			
Becalutamide	Monotherapy	II	NCT00468715 ([108])
Enzalutamide	Monotherapy	II	NCT01889238 ([106])
Orteronel	Monotherapy	II	NCT01990209
VEGF/VEGFR inhibitors			
Bevacizumab	Taxane therapy	III	NCT01094184
	Carboplatin/Gemcitabine	II	NCT01201265
	Abraxane	II	NCT00472693
	Carboplatin/Cyclophosphamide or Paclitaxel	II	NCT01898117
Ramucirumab	Capecitabine	II	NCT01234402
Apatinib	Monotherapy	II	NCT01176669 ([109])
Sunitinib	Monotherapy	II	NCT00246571
	Paclitaxel/Carboplatin	I/II	NCT00887575
Sorafenib	Cisplatin followed by Paclitaxel	II	NCT01194869
	Pemetrexed	II	NCT02624700
EGFR inhibitors			
Cetuximab	Cisplatin	II	NCT00463788 ([110])
	Carboplatin	II	NCT00232505 ([111])
	Ixabepilone	II	NCT01097642
			NCT00633464
Erlotinib	Bendamustine	I/II	NCT00834678

Table 2 (continued)

Targeted therapy	Therapeutic combinations	Clinical phase	ClinicalTrial.gov Identifier (Ref.)
	Monotherapy	II	NCT00739063
	Metformin	I	NCT01650506
Panitumumab	Paclitaxel/Carboplatin	II	NCT01009983
			NCT02593175
	Gemcitabine/Carboplatin	II	NCT00894504
HGFR inhibitors			
Tivantinib	Monotherapy	II	NCT01575522 ([112])
FGFR inhibitors			
Dovitinib (TKI-258)	Monotherapy	II	NCT00958971
NOTCH inhibitors			
RO4929097	Paclitaxel/Carboplatin	I	NCT01238133
	Monotherapy	II	NCT01151449
	Vismodegib	I	NCT01071564
JAK2 inhibitors			
Ruxolitinib	Monotherapy	II	NCT01562873
	Paclitaxel	I/II	NCT02041429
Cyclin-dependent kinases inhibitors			
Dinaciclib	Epirubicin	I	NCT01624441
P276-00	Gemcitabine/Carboplatin	I	NCT01333137
MEK1/2 inhibitors			
Trametinib (GSK1120212)	Akt Inhibitor GSK2141795	II	NCT01964924
	Monotherapy	II	NCT01467310
	BMK-120	I	NCT01155453

PI3K/Akt/mTOR inhibitors

Inhibitors targeting the major mediators in the PI3K/Akt/mTOR pathway have also reached clinical trials (Table 2). Several studies are ongoing to evaluate the combination of mTOR inhibitors, everolimus and temsirolimus (rapamycin analogues), with chemotherapy, platinum agents or other targeted therapies, in the setting of TNBC. Among these, everolimus was generally associated with more adverse effects and did not significantly improve clinical response rates [93, 94]. Nonetheless, a clinical benefit was demonstrated in a combination of everolimus and carboplatin [95].

In respect to PI3K inhibitors, these are relatively new within TNBC clinical landscape, but several compounds have been evaluated in phase I trials [96–99] (Table 2). Adding to the spectrum of agents targeting the PI3K pathway, a selective small molecule inhibitor of all three Akt isoforms, Ipatasertib, enabled a robust antitumor activity in patient-derived xenografts models [100]. Two clinical studies are ongoing to evaluate the efficacy of ipatasertib combined with paclitaxel in the treatment of early stage (FAIRLANE) [101] and metastatic (LOTUS) [102] TNBC patients. Targeting the PI3K signaling pathway might benefit the subset of TNBCs with mesenchymal/mesenchymal stem-like features [70, 73]. Additionally, PI3K suppression may confer sensitivity to PARP inhibition in TNBCs without *BRCA* mutations, by impairing homologous recombination in DNA repair [103]. This provides a new rationale to combine PI3K and PARP inhibitors in this indication.

Androgen receptor inhibitors

The androgen receptor, which has been implicated in breast cancer pathogenesis [104], is expressed in more than 70% of breast tumors, including triple-negative (35%), generating particular interest in this subset of patients [105]. The luminal androgen receptor subtype is heavily enriched in hormonally regulated pathways and, alike the luminal intrinsic subtype, they are less likely to benefit from the current chemotherapy regimens [62]. This suggests that these patients may also benefit from androgen receptor inhibitors or, eventually, a combination of androgen receptor/PI3K inhibitors [81, 80]. At present, three anti-androgens are being evaluated in TNBC (Table 2), with promising results coming from the treatment with single agent enzalutamide in advanced androgen receptor-positive TNBC [106]. Lastly, orteronel, a potent

inhibitor of 17,20-lyase enzyme, impaired androgen synthesis at the preclinical level [107], and is now under evaluation in androgen receptor-positive TNBC (Table 2).

VEGF/VEGFR inhibitors

VEGF is implicated as the major angiogenic factor in human cancers, contributing to tumor growth and metastases [113]. In fact, TNBCs have higher levels of VEGF than other breast tumors [114]. The use of anti-angiogenic therapies in TNBC was supported by the results from a phase III trial, in which the combination of bevacizumab with paclitaxel resulted in increased response rates and time to progression [115–117]. In the setting of metastatic TNBC, the RIBBON-2 trial showed marked improvements in progression-free survival with bevacizumab and a trend towards improved overall survival [118]. Nevertheless, preliminary results from a recently completed clinical trial reported no difference in overall survival upon the combined administration of bevacizumab with adjuvant chemotherapy. Moreover, the use of this monoclonal antibody was associated with increased incidences of grade 3 or worse hypertension, severe cardiac events, and treatment discontinuation [119]. In 2010, the Food and Drug Administration (FDA) withdrew the recommendation for the use of bevacizumab in the treatment of breast cancer due to safety concerns [120]. Nevertheless, bevacizumab and other agents targeting VEGF and its receptor are still being evaluated in multiple clinical trials (Table 2).

EGFR inhibitors

Similar to VEGF, the epidermal growth factor receptor 1 has been explored as a therapeutic target in breast cancer, and has long been associated with basal-like TNBC [28, 121]. Two completed clinical trials have assessed the combined use of cetuximab with platinum agents (Table 2). The association of cetuximab with cisplatin resulted in increased progression free survival and overall survival, but the overall response rate compared to cisplatin alone (20% vs 10%, respectively) did not reach statistical significance, failing the primary endpoint of the study [110]. In the TBCRC001 trial, limited activity was observed with the combination of cetuximab and carboplatin, despite EGFR pathway activation in most TNBC patients recruited for the study. This suggested the existence of alternative mechanisms for the pathway activation [111].

The combination of cetuximab with ixabepilone was also evaluated in early and advanced TNBC. In the first setting, the combination improved the rate of complete response in TNBC patients [122], while it presented a similar level of clinical activity compared to ixabepilone alone in the second case [123]. These results suggest that a better understanding of the pathways maintaining EGFR activity is still required. An analysis of two randomized phase II trial pointed to a high expression of *PTEN*, low expression of *CRYAB* and absence of *KRAS* amplification as potential predictive markers of cetuximab efficacy, in patients with basal-like breast tumors [124].

JAK2 inhibitors

JAK2 amplification have been identified in residual triple-negative tumors, following neoadjuvant chemotherapy [125]. Evidence from preclinical studies suggested that the JAK2/STAT3 pathway is preferentially active in a chemotherapy-resistant population of cancer cells. Its inhibition in mouse models resulted in impaired tumors growth [126]. Ruxolitinib, which is already approved for the treatment of patients with myelofibrosis, is currently being investigated for the treatment of triple-negative inflammatory breast cancer (Table 2).

Other targeted molecules

Several clinical trials in TNBC patients have evaluated or are currently addressing the therapeutic potential of small molecule inhibitors targeting other signaling pathways in TNBC involving MEK, HGFR, FGFR, NOTCH, or the cyclin-dependent kinases, listed in Table 2.

Targeting the immune system in triple-negative breast cancer

The ability of cancer cells to adapt and circumvent the immune system has long been recognized has a hallmark of cancer [127]. Thus, it is not surprising that immunotherapy emerged in the past few years as a therapeutic option for this disease. The expression of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death-1 (PD-1) receptor in T-cells, and the expression of programmed death-ligand 1 (PD-L1) in the tumor microenvironment, endows tumors with a mechanism to escape adaptive immunity through the disruption of T-cell checkpoint pathways [128]. Antibodies targeting these molecules have been able to reverse the inhibition of the acquired immunity, hence restoring anti-tumor T-cell activity, resulting in increased response rates and overall survival in patients with a broad range of tumor types [129–135].

In respect to breast cancer, the higher proclivity of the triple-negative subtype to produce neoantigens, arising from their increased genomic instability and mutational load, might result in higher susceptibility to immunotherapy [136]. Interestingly, TNBCs also present a higher content of lymphocytic infiltrations [137], and the expression of PD-L1 protein or mRNA was found prevalent in these patients [138–140]. Additionally, both tumor-infiltrating lymphocytes and PD-L1 expression were associated with therapeutic outcome. Loi and colleagues reported that each 10% increase in intratumoral and

stromal lymphocytic infiltrations was associated, respectively, with a 17% and 15% reduced risk of relapse, and 27% and 17% reduced risk of death in ER-negative/HER2-negative breast cancer, regardless of chemotherapy nature [137]. The expression of PD-L1 was also predictive of a better pathologic complete response to neoadjuvant chemotherapy [138, 140]. Altogether, the data supported the concept that a subset of patients with triple-negative tumors might benefit from immunotherapy, and suggested that immune modulation could improve the clinical outcome, when associated with immunogenic chemotherapeutics such as doxorubicin or cyclophosphamide [141, 142]. Following this rationale, numerous clinical trials (Table 3) has been designed to evaluate the therapeutic impact of T-cell checkpoint inhibitors in the setting of TNBC.

Table 3 reflects ongoing clinical trials that include only patients with TNBC or several cancer malignancies amongst which TNBC. In these studies, T-cell checkpoint inhibitors are being evaluated as monotherapy or in combination with chemotherapy or other small molecule inhibitors. The therapeutic advantages of introducing immune checkpoint inhibitors into treatment regimens has been previously established for other solid tumors [143]. These results are encouraging and forecast immune checkpoint inhibitors as a promising therapeutic strategy for the treatment of breast cancers with the triple-negative signature.

Table 3 Clinical trials evaluating T-cell checkpoint inhibitors in patients with triple-negative breast cancer

Targeted therapy	Clinical phase	ClinicalTrial.gov identifier
PD-1 inhibitors		
JS001	I	NCT03151447, NCT02838823
Pembrolizumab (MK-3475)	I	NCT03012230, NCT02622074, NCT02646748
	I/II	NCT02657889, NCT02513472
	II III	NCT02411656, NCT02447003, NCT02752685, NCT02648477
		NCT02555657, NCT02819518, NCT03036488
PDR001	I	NCT02890069
	I/II	NCT02404441, NCT02829723
Nivolumab	I	NCT02309177
PD-L1 inhibitors		
Avelumab	III	NCT02926196
Durvalumab (MEDI4736)	I	NCT02826434
	I/II	NCT02628132, NCT02489448, NCT02484404
	II	NCT02685059
Atezolizumab (MPDL3280A)	I	NCT02655822
	I/II	NCT02708680, NCT02543645
	II	NCT03164993, NCT02849496
	III	NCT02425891, NCT03125902
FAZ053	I	NCT02936102
CA-170	I	NCT02812875
CTLA-4 inhibitors		
Ipilimumab	I	NCT01986426
	I/II	NCT01928394
Tremelimumab	I	NCT02658214
	II	NCT02527434

Conclusion

The molecular complexity of breast cancer has changed our view about the biologic diversity of this disease and, particularly, altered the way clinical treatment decisions are taken. Both molecular and immunohistochemical panels of biomarkers are currently being applied to predict the benefit of specific therapies, such as, endocrine and HER2-targeted therapy. The potential benefit of breast cancer molecular dissection might be particularly relevant to a subset of women diagnosed with triple-negative breast cancer, who do not currently benefit from targeted therapies and are associated with poor prognosis. The somewhat disappointing results from these early clinical trials might be partially explained by the heterogeneity inherent to TNBC. Most of these studies were performed in a group of patients with unselected triple-negative tumors, *i.e.*, only selected based on the absence of ER, PR and HER2 by immunohistochemical characterization. However, heterogeneity of TNBC considerably contributes to dilute the effect of a treatment that otherwise could be effective in a molecularly selected subset of patients.

In spite of the considerable advancements in the treatment of breast cancer in recent years, many of these patients continue to progress to metastatic disease and, for those with advanced breast cancer, palliative care is oftentimes the endpoint. From a therapeutic standpoint, the significant molecular and genetic differences between primary cancers and their paired metastases, and potentially between metastases within the same patient, has been a challenge for the development of approaches to address metastatic disease. Perhaps the modulation of the immune response, through the combination of immune checkpoint inhibitors to molecularly targeted strategies, could potentiate the therapeutic outcomes. Nevertheless, these biological hurdles should be faced as an opportunity to find novel targets and develop targeted strategies addressing this problem.

Acknowledgements Ana Cristina Leal Gregório is student of the international PhD program in Experimental Biology and Biomedicine (PDBEB) from the Institute for Interdisciplinary Research, University of Coimbra and recipient of the fellowship SFRH/BD/51190/2010 from the Portuguese Foundation for Science and Technology (FCT). The work was supported by the grants PTDC/SAU-BMA/121028/2010 and POCI-01-0145-FEDER-007440 (FEDER/COMPETE 2020/FCT).

Compliance with Ethical Standards

Conflict of Interest Authors declare no competing financial interests.

Abbreviations *BL1*, basal-like 1; *BL2*, basal-like 2; *CTLA-4*, cytotoxic T-lymphocyte antigen 4; *ER*, estrogen receptor; *FDA*, food and drug administration, *HER2*, human epidermal growth factor receptor 2; *IM*, immunomodulatory; *LAR*, luminal androgen receptor; *LHRH*, luteinizing hormone-releasing hormone; *M*, mesenchymal; *MSL*, mesenchymal stem-like; *PARP*, poly (adenosine diphosphate-ribose) polymerase; *pCR*, pathologic complete response; *PD-1*, programmed death-1 receptor; *PD-L1*, programmed death-ligand 1; *PR*, progesterone receptor; *TNBC*, triple-negative breast cancer

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