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# **Current options in breast cancer targeted therapies: life after HER-2**

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## Abstract

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Breast cancer (BCa) is a major cause of cancer related morbidity and mortality. In advanced disease, therapeutic options are expanding rapidly. Avid targeted therapy research contributes decisively to this development and new agents are brought to light constantly. Trastuzumab is no longer the only option in estrogen receptor (ER) negative, epidermal growth factor receptor-2 (HER-2) positive BCa. Anti-angiogenic compounds like bevacizumab, as well as dual HER-1 and HER-2 tyrosine kinase inhibitor (TKi) lapatinib are now approved in BCa treatment and hold promise in the right subset of patients. Other emerging therapies include mammalian target of rapamycin inhibitors (mTORi), farnesyl transferase inhibitors (FTi), Histone deacetylase inhibitors (HDACi), poly (ADP-ribose) polymerase-1 inhibitors (PARP-1i) among others. Nonetheless, selection of the target, associated toxicities and cost/benefit ratio are questions that one should keep in mind when elaborating a therapeutical plan. This review attempts to critically summarize the published data on targeted therapies for breast cancer and respective biomarkers predictive of response.

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**Key words:** Breast cancer . Targeted therapies . HER-2 . Predictive biomarkers

### Epidemiology and clinical relevance

Breast cancer (BCa) is the most common cancer in women, accounting for 131 900 deaths in Europe yearly [1,2,3]. Worldwide it is estimated that 1.3 million women are diagnosed with BCa every year and 465 000 will die from the disease [3]. This heavy burden on public health is the driving force for intense research directed to this pathology in particular.

After more than twenty years of continuous increase in BCa incidence, we have witnessed an average 2.2% yearly decrease from 1999-2005 [4]. Mortality rates have also been decreasing over time, probably due to improvements on screening programs and therapeutical strategies.

Risk factors for developing BCa include gender, age, age at menarche, first live birth, personal, and familial history of breast cancer, certain benign breast conditions, and previous radiation exposure. Breast cancer is about 100 times more frequent in women than in men, not only because the mammary glands are more developed in women, but also because women's mammary cells are continuously exposed to the growth-promoting effects of estrogen and progesterone. BCa is uncommon before the age of 25 except in familial cases, and the incidence rises throughout a woman's lifetime with most cases occurring after the 6<sup>th</sup> decade of life. Women who reach menarche younger than 11 years old have a 20% increased risk when compared to women who had it after the age 14 years. Late menopause is also associated with an increased risk but the magnitude has not yet been precisely determined. Child delivery before the age of 20 years reduces the risk in half when compared to nulliparous women or women giving birth after

35 years of age. Importantly, previous personal history of BCa confers a 3 to 4 fold increase in risk of developing the disease. It is also important to assess familial cases of the disease although only about 13% of women with breast cancer have one affected first degree relative. Of those, 25% (3% of all breast cancer) are originated by two autosomal-dominant germ-line mutations: BRCA1 and BRCA2 which confer a lifetime risk of 60 to 80% as well as an augmented risk for other types of cancer such as ovarian, pancreatic, colon, and prostate cancers [5]. Certain benign breast conditions are associated with an increased risk of developing BCa, such as proliferative lesions with or without atypia, the former conferring a greater risk. Additional risk factors are recognized but lack definitive evidence or it is difficult to quantify their weight on the development of BCa. This category comprises physical exercise, diet, geographic influence, obesity, estrogen exposure, among others.

Owing to the morbidity and mortality associated with BCa, emphasis has been placed in early detection strategies. However, screening programs are not uniform across the globe, especially with respect to the age at which it should be started and to the periodicity of the test. In the EU, recommended screening for BCa consists on mammography plus clinical breast examination on middle aged women. In Portugal, recommendations to the general female population are one mammography every other year starting at 50 until 69 years old [6].

If detected early, BCa has a very good prognosis whereas advanced stage disease is associated with a dismal prognosis in spite of the different therapeutical options. In early stages, surgery followed of radiotherapy and/or chemotherapy is usually recommended with intent to cure the disease. In advance disease, palliative therapy is offered, based mainly on radiotherapy plus chemotherapy and/or hormonotherapy and targeted therapy.

### **Histologic and Molecular classification of Breast Cancer**

The vast majority of breast cancers are carcinomas with glandular/ductal differentiation, corresponding approximately to 95% of all BCa. Other less frequent types include squamous cell carcinoma, malignant phyllodes tumor, sarcoma, and lymphoma. Carcinomas are further divided into in situ carcinomas and invasive types. In situ carcinoma implies that tumor cells are confined to ducts and lobules without surpassing the basement membrane and, therefore, do not metastasize. On the contrary, invasive carcinomas, by definition, infiltrate the breast stroma and are able to invade lymph and blood vessels, reaching the lymph nodes and/or other distant sites. Both "in situ" and "invasive" types of breast carcinoma are further subdivided into subtypes, the most common of which are ductal carcinoma in situ, not otherwise specified (DCIS, NOS) and invasive ductal carcinoma of no special type (IDC, NST) which represents 70% of all BCa. IDC, NST is in itself an heterogeneous group, comprising well-differentiated tumors expressing hormone receptors without HER2 overexpression, and less differentiated cancers that overexpress HER-2 and do not express hormone receptors. Other types of invasive breast carcinoma include lobular, tubular, medullar, and other less frequent subtypes [7].

Irrespective of its histological subcategorization, breast carcinomas are further graded according to the Nottingham grading system [7]. This classification scheme evaluates the architectural and

cytological differentiation as well as the proliferative activity (Table 1), allowing for the stratification of patients in different groups with defined risk for tumor progression.

**Table 1 – The Nottingham grading system for breast carcinoma.**

<b>Nottingham criteria</b>			
	<b>Tubule Formation</b>	<b>Mitotic Count</b>	<b>Nuclear Pleomorphism</b>
<b>1</b>	Majority of tumor (>75%)	0-7 Mitoses/10 hpf	Small regular uniform cells
<b>2</b>	Moderate degree (10-75%)	8-16 Mitoses/10 hpf	Moderate nuclear size and variation
<b>3</b>	Little or none (<10%)	17 or > Mitoses/10 hpf	Marked nuclear variation

Each tumor is categorized according to a score derived from the sum of each of the three parameters, as detailed in the text. hpf: high power field.

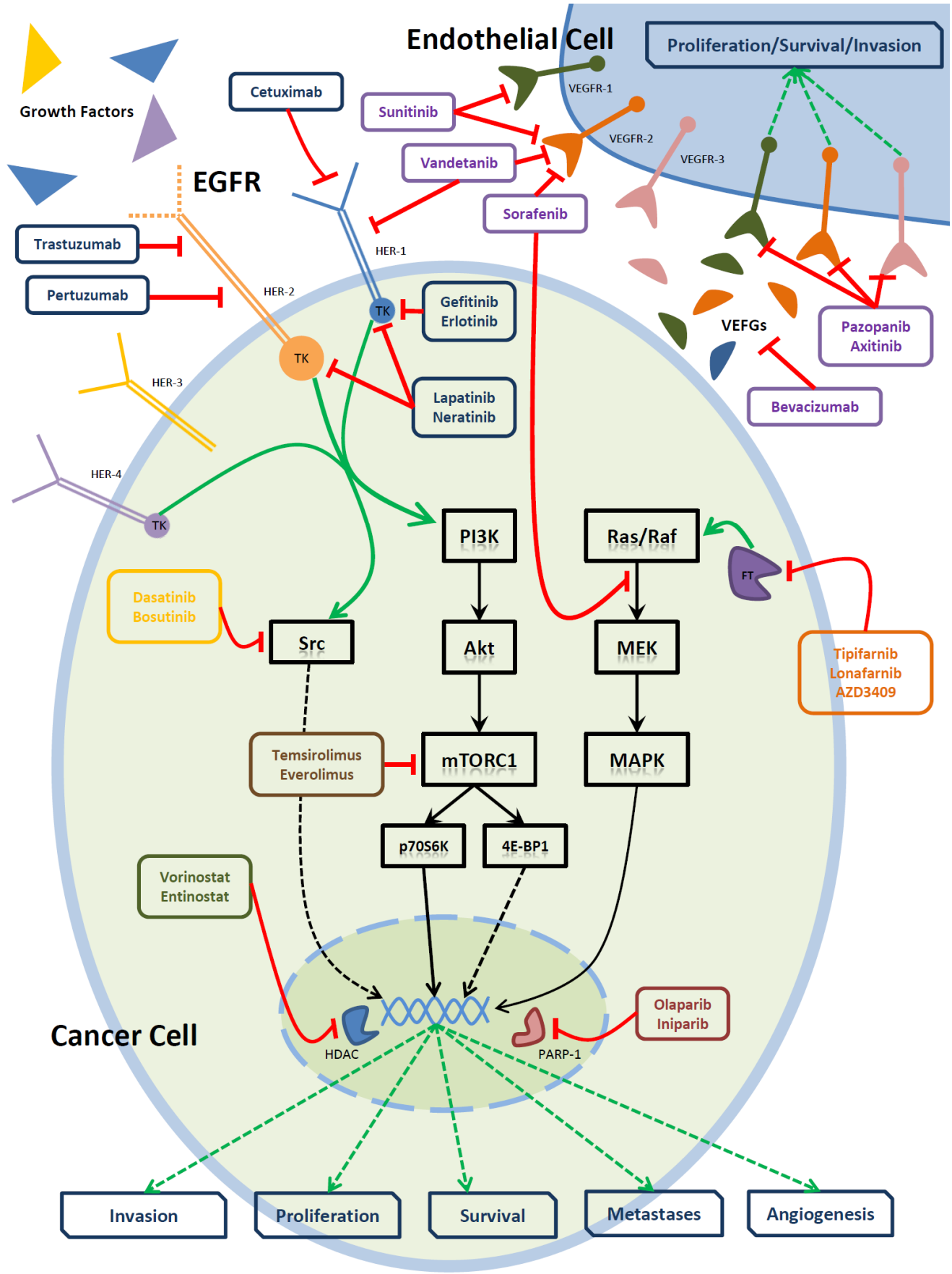
Tumors scoring between 3 and 5 (grade I) are well differentiated, whereas tumors with scores 6 and 7 (grade II), are moderately differentiated, and, finally, tumors scoring between 8 and 9, are poorly differentiated, high grade tumors.

The availability of high-throughput technologies which enable a fast genome-wide characterization of cancer cells created the possibility of defining molecular signatures that putatively correlate with clinical features and, importantly, with response to therapy. Indeed, efforts have been developed to categorize different types of BCa according to their molecular profile and correlated with the prognosis and prediction, to some extent, of the response to novel targeted therapies. One of the first approaches was to determine the hormone receptor [estrogen (ER) and progesterone (PgR) receptors] status in BCa patients. The most recent documents from the American Society of Clinical Oncology (ASCO) recommend that "ER and PgR should be measured on every primary invasive breast cancer and may be measured on metastatic lesions if the results would influence treatment planning" [8]. The current gold standard for this assessment is immunohistochemistry (IHC) performed on formalin-fixed, paraffin-embedded cancer tissues [9]. These results are widely used in the clinic to guide the therapeutical plan. HER-2 status is also currently tested in the same way. Present recommendations from ASCO state that "HER2 expression/or amplification should be evaluated in every primary invasive breast cancer either at the time of diagnosis or at the time of recurrence, principally to guide selection of trastuzumab in the adjuvant and/or metastatic setting" [8]. These evaluations constitute, today, the mainstays of BCa profiling and are of critical importance since the most frequently used target therapies at present time are the ER antagonists (e.g., tamoxifen) and the HER-2 antagonists (e.g., trastuzumab).

In recent years, a study examining gene-expression patterns of BCa suggested that at least four molecular classes could be defined: luminal-like, basal-like, normal-like, and HER-2 positive [10]. These classes have implications for choosing the molecular targeted therapy. For instance, luminal-like cancers tend to express ER and have low or intermediate grade whereas basal-like cancers tend to be ER negative and of high grade. Additional studies have shortened the list to three molecular classes: luminal-like (91% ER positive), basal-like (77% ER negative), and HER-2 positive (64% HER-2 amplified) [9]. In addition, luminal-like BCa is now subdivided into luminal A and luminal B. Luminal A includes predominantly ER positive and/or PgR positive and HER-2 negative tumors, whereas luminal B contains mainly ER positive and/or PgR positive and HER-2 positive tumors and/or Ki67 immunoreactivity in more than 14% of tumor cells. New biomarkers are being incorporated in order to categorize BCa more accurately. Not only ER, PgR, and HER-2 immunohistochemical evaluation, but also immunostains for cytokeratins 5/6, EGFR and Ki67 expression are now taken into account. This novel approach allows for an improved identification of basal-like BCa through IHC (100% sensitivity and 76% specificity). These tumors are most often, but not exclusively, triple negative tumors (*i.e.*, ER, PgR and HER-2 negative) and CK5/6 and/or EGFR positive [11].

### **Targeted therapy in Breast Cancer**

As stated in the previous section, one of the main driving forces behind the molecular characterization of BCa is the ability to define molecular targets with therapeutical usefulness. Targeted cancer therapies are based on compounds that block the growth and spread of cancer by interfering specifically with the cancer cell biology. In theory, this new approach is "smarter" than conventional chemotherapy regimens, since it focuses on the differences between cancer and normal cells. Dissimilarities in signal transduction, receptor expression, cell survival, and angiogenesis are among the ones explored in several studies. Potentially it holds less side effects and it is more effective than the standard "blind" chemotherapy. The first targeted therapy used in BCa was directed against estrogen receptors (ER). It is still widely used and it is responsible for major improvements in the cure rates for patients with ER positive breast tumors. Nowadays, numerous other targets have been investigated, such as the epidermal growth factor receptor (EGFR/HER) family, the vascular endothelial growth factor (VEGF), the mammalian target of rapamycin (mTOR), tumor necrosis factor-related apoptosis inducing ligand (TRAIL), inhibitors of farnesyl transferase (FTi), among others. These novel therapies require a wide understanding of the molecular mechanisms behind the altered "cell-machinery" and regulatory systems in neoplasms. For instance, it is known that breast cancer cells overexpress anti-apoptotic molecules and tend to inhibit or down regulate the pro-apoptotic ones, meaning that the balance between pro and anti apoptotic mechanisms is biased towards the survival of the cancer cell. In the following sections, we will approach the several targeted therapies available or under development for BCa, according to their class.



**Figure 1** - Major pathways involved in carcinogenesis and targets for therapy. EGFR/HER: epidermal growth factor receptor; VEGF(R): vascular endothelial growth factor (receptor); TK: tyrosine kinase; FT: farnesyl transferase; mTORC1: mammalian target of rapamycin complex-1; HDAC: histone deacetylase; PARP-1: Poly (ADP-ribose) polymerase-1.

## Targeted therapy by class

### Anti-EGFR agents

HER-2 belongs to the EGFR family, a group of tyrosine kinases involved in signal transduction, through Ras/mitogen-activated protein kinase pathway, PI3K/Akt pathway, Janus kinase/signal transducer and activator of transcription pathway, and the PLC- $\gamma$  pathway, ultimately leading to cell proliferation, survival, motility, and adhesion [12]. The signal transduction takes place when a ligand binds the receptor, causing dimerization and activation of the tyrosine kinase, which is responsible for the effective response. There are four types of EGFRs: HER-1, HER-2, HER-3 and HER-4. Hence, dimerization may occur with the same type of ligand, forming homodimers, or with a different type of ligand, resulting in heterodimer formation. The latter are more effective signal transducers, especially when HER-2 is involved [13]. Interestingly, HER-2 overexpression occurs in about 20-30 % of BCa and it correlates with poor prognosis. It has also an inverse relationship with estrogen receptor expression [13]. Furthermore, it has been associated with high tumor grade, DNA aneuploidy, high cell proliferation rate, p53 mutations, topoisomerase II $\alpha$  amplification and alterations in other molecular biomarkers of breast cancer [12].

### *Trastuzumab*

The prototypical anti-EGFR agent for breast cancer is trastuzumab. This is a monoclonal antibody that specifically binds the extracellular portion of HER-2. It was first launched to market in 1998, and it has become the most important therapeutical option for HER-2 overexpressing BCa [14]. It has demonstrated clinical usefulness as first, second and third-line treatment, in monotherapy or combined with standard chemotherapy or other targeted therapies [14]. Indeed, five randomized trials using trastuzumab in the adjuvant setting showed a considerable reduction of mortality ( $p < 0.00001$ ), recurrence ( $p < 0.00001$ ) and metastases ( $p < 0.00001$ ) rates when compared to patients which did not receive adjuvant trastuzumab. These results confirmed trastuzumab's indisputable role in BCa therapy. Furthermore, data from that meta-analysis were considered sufficient to issue the recommendation of one year of adjuvant trastuzumab in women with HER-2 positive early BCa [15,16]. However, these patients should however undergo a careful cardiac monitoring because cardiac toxicity is a serious side-effect, especially when trastuzumab is combined with antracyclines [16]. Fortunately, cardiac toxicity is reversible in most cases, and probably idiosyncratic instead of dose-dependent.

The prescription of trastuzumab implies that the tumor overexpresses HER-2, and this condition may be determined by immunohistochemistry and/or in situ hybridization (fluorescence or chromogenic-based, i.e., FISH or CISH). Usually, immunohistochemistry for HER-2 is initially performed to categorize tumors as negative (immunostains scores 0 and 1+), positive (3+) or equivocal (2+)/inconclusive [17]. The latter cases are then directed to FISH analysis to determine whether there is HER-2 gene amplification or not. Only cases positive by immunohistochemical analysis or with gene amplification by FISH/CISH are amenable to therapy with trastuzumab.

However, only about 50% of those patients will actually respond to trastuzumab. Thus, alternative targeted therapies are required for this subgroup of BCa patients.

### ***Lapatinib***

Lapatinib is an oral dual tyrosine kinase inhibitor (TKi) that targets both HER-1 (EGFR) and HER-2. It is currently approved in combination with capecitabine by the EMEA and the FDA for HER-2 positive, previously treated (after anthracyclines, taxanes and trastuzumab) metastatic breast cancer (MBCa) patients. Lapatinib was well tolerated and no serious adverse reactions were accounted in phase I studies [18]. As single agent in HER-2 overexpressing advanced or MBCa that progressed on trastuzumab-containing regimens, complete response (CR) was reported in 7.7% of the seventy-eight patients enrolled and a partial response (PR) in 5.1%, with a median time to progression (MTP) of 15.3 weeks and a median overall survival (MOS) of 79 weeks [19]. Several phase III trials demonstrated the effectiveness of lapatinib in combination treatments [20,21]. One of these studies showed a longer time to progression (TTP) ( $p < 0.001$ ) and a non-statistically significant improved overall survival (OS) ( $p = 0.177$ ) in the capecitabine plus lapatinib arm when compared to capecitabine plus placebo [20]. A recent meta-analysis of randomized phase III trials in MBCa determined that the use of lapatinib should be confined to patients with HER-2 positive breast cancer because its activity is limited to this cluster of patients [22]. Unlike trastuzumab, lapatinib is able to cross the blood-brain barrier making it a possible candidate for the treatment of brain metastases. In fact, a recent study from the UK, points out that lapatinib plus capecitabine is a valid therapeutical option in women with brain metastases that exhausted all therapeutic alternatives (anthracyclines, taxanes, trastuzumab) [23]. Moreover, cardiac toxicity is rare and not as severe as reported for trastuzumab [24,25].

Consistent with results from the studies mentioned above, HER-2 overexpression is positively correlated with response to lapatinib [26,27] and remains the only established biomarker to the present date. Interestingly, it has been suggested that increased levels of phosphorylated HER-2 (pHER-2) and HER-3 (pHER-3) also correlate with clinical benefit of lapatinib in patients with inflammatory breast cancer (IBCa) [28].

### ***Pertuzumab***

Pertuzumab is a humanized monoclonal antibody that binds HER-2 receptor and disrupts HER-2 heterodimerization. Interestingly, pertuzumab couples with a different subdomain (II) of HER-2 which makes it, in theory, a synergic partner of trastuzumab (which couples with subdomain IV) [29]. This association strongly enhanced antitumor activity in human xenograft models by activating antibody-dependent cellular cytotoxicity [30]. A phase II trial showed the combination to be well tolerated and active in MBCa [31] but limited to HER-2 overexpressing tumors. Cardiotoxicity, although generally asymptomatic, was reported in some patients [33]. A phase III trial is now underway to evaluate the therapeutic efficacy of the association of pertuzumab with trastuzumab plus docetaxel.

### ***Neratinib***

Neratinib, a selective and irreversible tyrosine kinase inhibitor of HER-1 and HER-2 (and eventually HER-4) has shown potential antitumor activity in HER-2 positive advanced breast cancer [34]. Various combinations were studied in phase I/II trials. Neratinib was associated with trastuzumab, with paclitaxel and with vinorelbine, each combination at a time, in different studies. In all of them, therapeutic activity of the association was demonstrated in MBCa [35,36,37]. Moreover, two phase III trials are currently active: one compares neratinib plus paclitaxel vs. trastuzumab plus paclitaxel as first line treatment for HER-2 positive locally recurrent or MBCa, and the other compares neratinib vs. lapatinib plus capecitabine in a similar setting [38].

### ***Gefitinib and Erlotinib***

Gefitinib and erlotinib are HER-1 selective tyrosine kinase inhibitors. Both agents have been approved for local advanced or metastatic non-small cell lung cancer (NsCLCa), which constitutes their major therapeutical indication. Studies in BCa showed very limited and modest anti-tumor activity [39,40]. Consequently, the development of gefitinib for BCa has been discontinued.

### ***Cetuximab***

Cetuximab, a monoclonal antibody that blocks HER-1 activation and consequent HER dimerization, has shown modest results in phase I/II studies. In a phase I trial, cetuximab plus paclitaxel disclosed prohibitive dermatologic toxicity and the preliminary results were disappointing [41]. When added to carboplatin for treatment of metastatic, triple-negative BCa patients, weak anti-neoplastic activity was observed [42]. Despite these results, some predictive biomarkers of response have been identified for the use of cetuximab, which may prove useful for the selection of patients which might profit the most from this agent. For instance, low expression of alpha basic crystallin (CRYAB) gene, high expression of PTEN and, in basal-like carcinomas, lack of KRAS amplification, were positively correlated with clinical response [43].

### **Anti-VEGF/VEGFR agents**

The vascular endothelial growth factor (VEGF) is a protein that actively binds the VEGF receptors (VEGFR) of endothelial cells in arteries, veins and lymph vessels. Until now, seven types of VEGF ligands (from A to F, and placental growth factor) and three VEGF receptors (from 1 to 3, which bind different VEGF ligands) have been recognized [47]. When a VEGF ligand attaches to its receptor, activation of endothelial tyrosine kinases (TK) ensues, leading to a biological response comprising proliferation, increased cell survival, vascular permeability, migration, and invasion [48]. VEGF expression is boosted by many factors such as hypoxia, epidermal growth factors, insulin-like factor 1 (IGF-1), among others. Overexpression of VEGF is correlated with poor prognosis in BCa patients [48].

Current targeted therapies aim either at the neutralization of VEGFR ligands, or the inhibition of VEGFR TK.

### ***Bevacizumab***

Bevacizumab is a humanized monoclonal antibody that neutralizes all main isoforms of VEGF by binding VEGFs and therefore preventing activation of VEGFRs. As single agent, the results of phase I/II trials in patients with MBCa indicated a 6.7% overall response rate (ORR) and OS of 10.2 months [49]. Another phase II trial evaluated the use of the HER-2 antagonist trastuzumab plus bevacizumab as first line treatment in HER-2 amplified advanced breast cancer. The association proved clinically feasible and very active even in the absence of concomitant chemotherapy [50]. When used in combination regimens, bevacizumab plus chemotherapy was compared to chemotherapy alone in HER-2 negative MBCa, in a randomized, double blind phase III trial. The chemotherapy regimen included capecitabine, anthracycline and taxanes. The results of this study showed a significant increase in progression free survival (PFS) in the combination regimen vs. chemotherapy alone [51]. In another phase III trial, 736 patients were randomized to receive docetaxel either with placebo or bevacizumab at 7.5mg/kg or 15mg/kg. Both bevacizumab arms demonstrated a significant increase in PFS in comparison with the placebo arm. The ORR was also increased (55% and 63% vs. 44%,  $p=0.0295$  and  $0.0001$ , respectively) [52]. In these two studies, bevacizumab was used as first line treatment. More recently, a randomized, double-blind phase III trial evaluated the efficacy and safety of bevacizumab in combination with chemotherapy as second line treatment in HER2-negative MBCa. The addition of bevacizumab to chemotherapy resulted in a significant improvement in PFS although no significant increase in OS was observed [53]. Recently, the Portuguese drug and health product authority (INFARMED) declined the approval of bevacizumab as a first-line agent in metastatic BCa when used in combination, owing to the lack of a significant increment in overall survival and quality of life, according to its interpretation of available data [54].

No predictive biomarker for response to bevacizumab has yet been found. Notwithstanding, some colorectal cancer studies attempted to correlate high levels of circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) with an improved clinical response to bevacizumab [55, 56].

### ***Sunitinib***

Sunitinib is a broad-spectrum VEGFR tyrosine kinase inhibitor, especially for VEGFR-1 and VEGFR-2. It has also inhibitory activity on c-KIT, FLT3, and platelet-derived growth factor receptors (PDGFRs). It is currently approved for treatment of metastatic renal cell carcinoma (as first line) and of gastrointestinal stromal tumors (GIST) unresponsive to imatinib. Concerning its efficacy in BCa, available data is inconsistent and contradictory.

As single agent, sunitinib exhibited antitumor activity in pretreated MBCa patients [57]. However, a randomized phase III trial evaluating the use of single agent sunitinib versus single agent capecitabine in advanced breast cancer (ABCa) patients was terminated due to futility [58]. Indeed, sunitinib was not superior to capecitabine and held more adverse reactions [58]. When in combination with paclitaxel, sunitinib showed preliminary antitumor activity and no drug-drug interaction was found. Interestingly, clinical responses were detected in patients with triple-negative receptor status (three out of nine) [59]. On the other hand, SABRE-B, a trial designed to evaluate paclitaxel and bevacizumab with or without sunitinib as first-line treatment of MBCa, proved the association to be unfeasible, due to severe toxicity in the combination regimen [60].

To date, a few biomarkers predictive of response for sunitinib were identified, although most of them in the context of treatment of renal cell carcinoma. One study suggested that overexpression of VEGF121 and VEGF165 (isoforms of VEGF-A) were correlated with better clinical response to sunitinib [61]. Other potential biomarkers include high levels of sVEGFR-3 and s-KIT [62].

### ***Sorafenib***

Sorafenib is another multi-kinase inhibitor of VEGFR-2, PDGFR, and Ras/Raf/MEK/ERK pathway through Raf kinase [48]. It is approved for the treatment of advanced renal cell carcinoma and unresectable hepatocellular carcinoma [48]. In BCa research, preclinical models, such as MDA-MB-231, exposure to sorafenib led to tumor regression only after nine days of treatment [63]. A double blind, randomized phase 2b study compared sorafenib plus capecitabine versus placebo plus capecitabine in ABCa patients. The oral combination demonstrated better PFS, it was well tolerated and no intractable toxicities were found [64]. Another phase II trial combined sorafenib with anastrozole with an intent to overcome resistance to aromatase inhibitors (AI), in ER positive patients, AI resistant MBCa. The association proved to have a clinical benefit of 20% which encouraged further studies in this field, and suggest a role for the Ras/Raf/MAPK pathway in AI resistance [65].

### ***Vandetanib***

Vandetanib is a dual inhibitor of VEGFR-2 and EGFR (HER-1). It showed limited activity as a single agent in MBCa patients with previous chemotherapy [66]. One study comparing vandetanib plus docetaxel with placebo plus docetaxel failed to demonstrate any benefit for the combination [67]. Despite the modest benefit of vandetanib in BCa therapy, lower baseline levels of VEGF seem to be positive predictors of response [68], which in due time might prove helpful for selecting patients for vandetanib therapy.

### ***Pazopanib and Axitinib***

Pazopanib and Axitinib are new multi-targeted TK inhibitors of VEGFRs. Their effects extend to PDGFR and c-KIT [14].

Pazopanib is well tolerated and demonstrates activity in pretreated breast cancer [69]. When used in combination with lapatinib, it demonstrated a superior response rate (36.2% vs. 22.2%)

indicating a synergistic effect of the two compounds [70]. Furthermore, it has been recently FDA - approved for the treatment of metastatic renal cell carcinoma.

Axitinib has been evaluated in a double-blind phase II trial, combined with docetaxel vs. docetaxel with placebo. Median TTP was longer in the combination arm (8.2 months vs. 7 months,  $p=0.052$ ). ORR was higher as well (40% vs. 23%,  $p=0.038$ ). Interestingly, subgroup analysis of patients previously treated with chemotherapy showed enhanced TTP and ORR (TTP: 9 months vs. 6.3 months,  $p=0.012$ ; ORR: 45% vs. 13%,  $p=0.003$ ). Axitinib plus docetaxel has anti-tumor activity and an acceptable safety profile especially in patients that underwent previous chemotherapy [71].

### **mTOR inhibitors**

The mammalian target of rapamycin (mTOR) holds a major role in cell processes such as growth, proliferation, and survival. This occurs mainly through two distinctive ways: by the activation of p70 s6 kinase, which leads to production of hypoxia-inducible factor 1a (HIF-1a), stimulating cell growth and angiogenesis, and by phosphorylating 4E-binding protein-1 that ultimately promotes expression of cell cycle stimulators such as c-myc, cyclin D1 and ornithine decarboxylase [73]. Many growth factors, as well as estrogen, cytokines and integrins are involved in mTOR regulation through PI3K/Akt signaling pathway [72].

At present, there two main drugs under research that antagonize mTOR: temsirolimus and everolimus [14]. In fact, one study reported that rapamycin combined with trastuzumab has produced better results than trastuzumab alone in HER-2 overexpressing BCa [74] reinforcing the use of rapamycin analogues in BCa targeted therapy.

#### ***Temsirolimus***

Temsirolimus has shown anticancer activity in BCa but also in other solid tumors, in particular renal cell carcinoma. The underlying mechanism of action of temsirolimus resides on the formation of a complex with FK 506-binding protein which ceases activation of mTOR. This complex affects only one subpopulation of mTOR proteins termed mTORC1, holding no effect in a second subpopulation known as mTORC2 [73].

A phase II trial tested the efficacy of temsirolimus as single agent in women with advanced BCa previously treated with taxanes and/or anthracyclines. Of the 109 patients, 10 (9.2%) had a partial response (PR) and stable disease (SD) was observed in 5 (4.6%) patients. Thus, clinical benefit (PR+SD) was achieved in only 15 patients (13.6%) [75,76]. Another phase II study suggested that PFS was longer in a combination of temsirolimus with letrozole than using letrozole alone (13.2 months versus 11.6 months) [77]. These results seem modest, probably due to shortage of predictive biomarkers, which in turn would make possible a more selective use of this class of agents. Nonetheless, some literature suggests that decreased phosphate and tensin homologue tumor suppressor (PTEN) gene expression as well as overexpression of phospho-s6 kinase (pS6K)

and pAkt are correlated with an increased clinical benefit from temsirolimus in the context of renal cancer [78].

### ***Everolimus***

Everolimus is another rapamycin analogue that showed promising results in a phase II randomized study comparing everolimus plus letrozole versus letrozole plus placebo in ER positive BCa. The response rate determined by clinical palpation was higher in the combination arm (68.1% vs. 59.1%), and a dramatic downregulation of ps6k was observed in the everolimus arm only [79]. These results suggested that everolimus may in fact, enhance the therapeutical activity of letrozole. Moreover, both agents, temsirolimus and everolimus, are involved in overcoming endocrine resistance in certain types of endocrine resistant BCa [80].

### **Farnesyl Transferase Inhibitors (FTi)**

Farnesyl transferase catalyses a critical step in Ras activation, *i.e.*, the covalent binding of a farnesyl isoprenoid lipid to a cysteine residue in the Ras protein (farnesylation). Thus, farnesylation inhibition ultimately prevents the activation of Ras-dependent mechanisms for cell survival and proliferation.

A few farnesyl transferase inhibitors are currently under investigation for BCa treatment, including tipifarnib, lonafarnib and AZD3409.

### ***Tipifarnib***

In phase I studies, tipifarnib demonstrated dose-related toxicities, such as diarrhea, nausea, renal dysfunction and myelosuppression [81]. Another study suggested that the addition of FTIs to tamoxifen or other estrogen receptor antagonists has a synergistic effect, encourage further investigations [82]. Combining tipifarnib with neoadjuvant doxorubicin-cyclophosphamide in stage IIB-IIIC BCa patients enhanced the pathologic complete response rate at surgery and downregulated p-STAT-3, thus potentiating doxorubicin cytotoxicity [83]. On the other hand, a randomized phase II trial which associated tipifarnib and letrozole in ER positive, postmenopausal women with ABCa, reported no benefit or improvement in objective response rate in this population of patients [84]. More recently, a phase II study demonstrated that tipifarnib plus fulvestrant in ER positive, AI resistant MBCa improved the clinical benefit rate from 32% (for fulvestrant alone) to 48% (in the combination regimen) [85].

### ***Lonafarnib***

According to phase I studies, lonafarnib does not exhibit farnesyl transferase inhibition at the maximum tolerated dose of 75 mg. Indeed, the contribution of lonafarnib to gemcitabine plus cisplatin anti-tumor activity was questionable and substantial toxicity was found [86]. However,

phase II studies using lonafarnib in combination with AI or trastuzumab plus paclitaxel are currently underway.

### ***AZD3409***

AZD3409 is a novel dual prenyltransferase inhibitor that showed activity against both farnesyl transferase and geranylgeranyl transferase. Preclinical studies demonstrated that AZD3409 significantly inhibited growth of gefitinib-resistant BCa cells [88]. Further studies are warranted to confirm its activity and to evaluate the efficacy of combination regimens.

### **Src Inhibitors (Srci)**

Src is a member of the Src family kinases (SFK) and a nonreceptor tyrosine kinase protein with important functions which include regulation of cell growth, proliferation, angiogenesis, invasion, and metastasis. In addition, it has a role in osteoclastic activation and bone resorption. It was found to be anomalously activated in the setting of bone metastases and in breast cancer tissue [89]. Many cytoplasmatic factors can trigger Src, including integrins and the HER family. Various Src inhibitors are being tested, the most developed of which is dasatinib.

### ***Dasatinib***

Dasatinib is presently approved for second-line treatment of chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. Breast cancer studies yielded encouraging results in combination with capecitabine [90], and other associations with letrozole, bevacizumab and paclitaxel are ongoing. Interestingly, a six-gene panel (ANXA1, CAV1, CAV 2, EPHA2, PTRF, and IGFBP2) was developed to predict sensitivity to dasatinib. Remarkably, the subset of BCa expressing dasatinib sensitive signature includes the basal-like subtype, thus suggesting that Srcis are a potential new therapeutic option for this poor-prognosis class of BCa [91].

### ***Bosutinib***

Previously denominated SKI-606, this novel compound suppresses Src and Abl protein kinases [92]. Treatment of breast cancer cells MDA-MB-231 with bosutinib led to a significant inhibition of cell proliferation, invasion and migration [93]. Another study corroborated these results, reporting that at concentrations of 250nmol/L, disruption of migration and invasion in breast cancer cell lines occurred. This dosage, however, was not sufficient to block cell proliferation and survival [94]. The combination with letrozole or capecitabine is being evaluated in ongoing clinical trials [95].

### ***Sacaratnib (AZD0530)***

Sacaratnib is a recent oral Src and Abl antagonist with high affinity and specificity to the tyrosine kinase domain [89]. Although studies about this agent in BCa are scarce, interest has been taken to

explore its effectiveness preventing acquired endocrine resistance in BCa [97,98]. A biomarker was found to predict response to sacaratinib, although in the pancreatic cancer context [99]. Expression of a pair of genes (LRRC19 and IGFBP2) identified by K-TSP classifier was highly correlated with tumor's sensitivity to sacaratinib [99].

### **Histone deacetylase inhibitors (HDACi)**

Histone deacetylases (HDACs) are a key component of the epigenetic machinery that controls chromatin modification and gene expression. HDACs also regulate functions of non-histone proteins through removal of acetylated lysine residues [100]. Together with histone acetyltransferases (HATs), they represent the two enzymatic families which are responsible for the control of histone-dependent DNA transcription [101]. Eighteen different histone deacetylases are recognized and no redundant functions have yet been found [102]. The classification of HDACs is based on the homology with yeast proteins. Class I includes, HDACs 1, 2, 3 and 8, and Class II HDACs 4, 5, 7 and 9. The remaining HDACs are distributed by other classes or subclasses [102]. Abnormal activity of HDACs is related to aberrant cell cycle, differentiation, and carcinogenesis [103]. In fact, inhibition of HDAC leads to cell growth arrest and activation of apoptosis [102], thus supports a role for HDACi in cancer therapy.

#### ***Vorinostat***

Vorinostat is paninhibitor of class I and II HDACs. It was the first of its class to be approved for the treatment of cutaneous T-cell lymphoma. Phase II trials showed that vorinostat added no clinical benefit when used as single-agent in relapsed or refractory BCa [104]. In another phase I/II trial, vorinostat improved effectiveness of paclitaxel and bevacizumab. In addition, it inhibited heat shock protein 90 (Hsp90) and downregulated Akt in BCa cells *in vivo* [105]. Recently, it was suggested that vorinostat could increase radiosensitivity of BCa. A brain metastasis model using MDA-MB-231-BR cells revealed the association of vorinostat (V) with radiotherapy (R) to be well tolerated and hold longer median survival (Controls=13d, V=16d, R=16d, V+R=27d, p=0.038) [106].

#### ***Entinostat***

Entinostat is a class I selective HDACi currently in phase II development for BCa and non-small cell lung cancer [108]. This compound induced ER expression and inhibited proliferation in triple-negative BCa. Furthermore, it has a synergistic effect with AIs in blocking growth in ER negative tumor cells [109,110]. In primary tumor models this efficacy in triple negative BCa was confirmed [108]. The association with lapatinib is currently being tested in inflammatory BCa [110].

### **TRAIL receptor agonists (TRAIL-Ra)**

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) superfamily of cytokines, and it is implicated in programmed cell death [111]. The respective receptor, TRAIL-R, preferably induces apoptosis in cancer cells with minimal effects

on normal cells. There are five receptors for TRAIL, but only two - TRAIL-R1 and TRAIL-R2 - effectively transduce the apoptotic signal [111]. The underlying mechanism is complex and involves formation of a trimerized receptor plus a Fas-associated death domain and caspase 8 or 10 [112]. Once active, caspase 8 (or 10) proceeds the cascade triggering apoptosis. Various TRAIL-R agonists have been investigated but all are still in an early phase of development.

### ***Mapatumab***

Mapatumab is a human monoclonal antibody, TRAIL-R1 agonist, currently in phase I/II studies. It was well tolerated with no prohibiting toxicities detected as single agent or in combination with gemcitabine and cisplatin. Preclinical activity has been observed [113,114]. Further studies are warranted.

### ***Apomab and Lexatumumab***

Both apomab and lexatumumab are TRAIL-R2 agonists. Apomab demonstrated potent antitumor properties both *in vitro* and *in vivo* [116]. Lexatumumab showed to be safe and well tolerated in pharmacokinetic, phase I studies [117,118].

### **PARP-1 inhibitors (PARP-1i)**

Poly (ADP-ribose) polymerases play important roles in regulation of the inflammatory process, genome repair mechanisms, and cell death [119]. Due to their vital role in cell biology, disruption of its normal activity leads to failure in DNA repair and, consequently, cellular collapse. This is the rationale behind the use of PARP inhibitors, in particular for PARP-1i (since it is most abundant PARP nuclear protein) as anticancer drugs [119]. In addition, PARP inhibition sensitizes p53 deficient BCa cells to doxorubicin-induced apoptosis and selectively kills cells with mutated BRCA1 and BRCA2 [120], which potentially offers a new option for treatment of hereditary forms of BCa.

### ***Olaparib***

Olaparib, a PARP-1 inhibitor, has anti-tumor activity in breast and ovarian cancers with BRCA1 and BRCA2 mutations without any major side effects [121]. After a phase I study identified 400 mg bid to be the maximum tolerated dose, a phase II trial assessed olaparib's efficacy, safety and tolerability in BRCA deficient ABCa patients. Olaparib proved to be well tolerated and highly active in this subset of patients [122].

### ***Iniparib (BSI-201)***

Iniparib is a new, potent and long-lasting PARP1-i. A randomized phase II trial combined iniparib with gemcitabine and carboplatin for treatment of triple negative MBCa. Results showed a remarkable improvement in clinical benefit rate, PFS and OS for the combination arm [123]. A phase III trial testing the same combination is currently active [124].

### Other targeted therapies

For the sake of completeness, other classes of targeted therapies in BCa deserve a brief mention. These include proteasome inhibitors, insulin-like growth factor receptor-1 (IGFR-1) antagonists and Hsp 90 inhibitors.

The proteasome is the final effector of the ubiquitin-proteasome pathway and plays a chief role in cellular homeostasis. Proteasome inhibition results in excessive accumulation of intracellular proteins which cause termination of the cell cycle or apoptosis [125]. The observation that neoplastic cell were more sensitive to proteasome inhibition, provided validation for further studies [126]. Bortezomib is a selective proteasome inhibitor approved for multiple myeloma treatment. In BCa, it has shown mediocre activity as single agent [127] and fair activity in association with capecitabine in heavily pretreated patients [128]. Still, there are multiple studies ongoing exploring bortezomib in combination regimens [129].

High levels of insulin-like growth factor 1 (IGF-1) as well as some polymorphisms of IGF-1 are correlated with a higher risk for developing BCa. Its receptor, the IGFR-1, is expressed in various tumors and its signaling is essential for tumor transformation and survival [131]. Cixutumumab, a novel monoclonal antibody that binds IGFR-1 has shown striking antitumor properties *in vitro* and enhanced activity of cytotoxic agents [132]. Several trials exploring the association of cixutumumab with capecitabine, temsirolimus and gemtacinabine are underway [133].

Heat shock protein 90 is an abundant chaperone protein essential for many cellular processes and maintenance of hormone receptors, kinases and other proteins. It is required for stability and activity of HER-2 and other key enzymatic complexes that promote cell survival and proliferation [134]. Inhibition of Hsp90 produces growth arrest and induces apoptosis, therefore constituting a target for novel therapies [135]. Tanespimycin is an Hsp 90 inhibitor that achieved good results in combination with trastuzumab. No severe toxicities were accounted [136]. However, additional studies are warranted to confirm its efficacy and safety.

**Table 2 – Targeted therapies for breast cancer**

<b>Class</b>	<b>Agent</b>	<b>Development Stage</b>	<b>Target</b>	<b>Predictive biomarkers</b>	<b>References</b>
<b>Anti-EGFR</b>	Trastuzumab	IV	HER-2 receptor	↑HER-2, ↑ <i>c-myc</i>	[14,15,16,45]
	Lapatinib	IV	HER-1 and HER-2 TKs	↑HER-2, ↑pHER-2, ↑pHER-3	[18,26,27,28]
	Pertuzumab	III	HER-2 receptor	↑HER-2 (?)	[30,32]
	Neratinib	III	HER-1 and HER-2 (HER-4) TKs	↑HER-2 (?)	[34,35]
	Gefitinib	Discontinued	HER-1 TK	-	[40,44]
	Erlotinib	II	HER-1 TK	↑HER-1 (in NsCLCa)	[39,46]
	Cetuximab	II	HER-1	↑PTEN, ↓CRYAB, [wild-type KRAS (in bIBCa)]	[41,43]
<b>Anti-VEGF/VEGFR</b>	Bevacizumab	IV	Pan-VEGF	[↑CECs (?), ↑CEPs (?)] (in CrCa)	[47,55,56]
	Sunitinib	III	VEGFR-1 and 2, c-KIT, FLT3, PDGFR	VEFGA (VEGF121, VEGF165) (in RCC), ↑s-KIT (?), ↑sVEGFR-3 (?)	[61,62]

**Table 2 – Targeted therapies for breast cancer (continued)**

<b>Class</b>	<b>Agent</b>	<b>Development Stage</b>	<b>Target</b>	<b>Predictive biomarkers</b>	<b>References</b>
<b>Anti-VEGF/VEGFR (continued)</b>	Sorafenib	I/II	VEGFR-2, PDGFR, RafK	None to date	[48, 65]
	Vandetanib	II	VEGFR-2, HER-1, RET	↓VEGF (?)	[66,67,68]
	Pazopanib	II/III	Pan-VEGFR, PDGFR, c-KIT	None to date	[69,70]
	Axitinib	I/II	Pan-VEGFR, PDGFR, c-KIT	None to date	[71]
<b>mTORi</b>	Temsirolimus	III	mTORC1	↓PTEN (?), ↑pS6K (?), ↑pAkt (?) (in RCCa)	[73,78]
	Everolimus	II/III	mTORC1	None to date	[79,80]
<b>FTi</b>	Tipifarnib	II	Farnesyl transferase	↑Ki67 (?), wild-type Ras (?)	[83,87]
	Lonafarnib	II	Farnesyl transferase	wild-type Ras (?)	[87]
	AZD3409	I	Farnesyl transferase and geranylgeranyl transferase	None to date	[88]

**Table 2 – Targeted therapies for breast cancer (continued)**

<b>Class</b>	<b>Agent</b>	<b>Development Stage</b>	<b>Target</b>	<b>Predictive biomarkers</b>	<b>References</b>
<b>Srci</b>	Dasatinib	II	SFK	Six-gene panel (see text)	[89], [91]
	Bosutinib	II	SFK	CAV-1 (?) (in PcCa)	[89], [96]
	Saccharatinib (AZD0530)	II	SFK	Two genes (↑LRRC19 and ↑IGFBP2) (in PcCa)	[99]
<b>HDACi</b>	Vorinostat	I/II	HDAC class I and II	↑HR23B (in CTCL)	[107]
	Entinostat	I/II	HDAC class I	None to date	[107,108]
<b>TRAIL-Ra</b>	Mapatumumab	I/II	TRAIL-R1 (DR4)	None to date	[114,115]
	Apomab	I	TRAIL-R2 (DR5)	None to date	[116]
	Lexatumumab	I	TRAIL-R2 (DR5)	None to date	[117,118]

**Table 2 – Targeted therapies for breast cancer (continued)**

<b>Class</b>	<b>Agent</b>	<b>Development Stage</b>	<b>Target</b>	<b>Predictive biomarkers</b>	<b>References</b>
<b>PARP-1i</b>	Olaparib	II	PARP-1	Mutated BRCA 1/2 (?)	[120,121]
	Iniparib (BSI-201)	II/III	PARP-1	None to date	[123,124]
<b>Proteasome inhibitors</b>	Bortezomib	II	Proteasome	↑Cyclin D1	[130]
<b>IGFR-1 antagonists</b>	Cixutumumab	I/II	IGFR-1	None to date	[132]
<b>Hsp 90 inhibitors</b>	Tanespimycin	I/II	Hsp 90	None to date	[136]

**HER:** epidermal growth factor receptor; **TK:** tyrosine kinase; **NsCLCa:** non-small cell lung cancer; **bIBCa:** basal-like breast cancer; **VEGF:** vascular endothelial growth factor; **CEC:** circulating endothelial cells; **CEP:** circulating endothelial progenitors; **CrCa:** Colorectal cancer; **(s)VEGFR:** (soluble) vascular endothelial growth factor receptor; **PDGFR:** platelet-derived growth factor receptor; **mTORi:** mammalian target of rapamycin inhibitor; **RCCa:** renal cell carcinoma; **mTORC1:** mammalian target of rapamycin complex 1; **FTi:** farnesyl transferase inhibitor; **Srci:** Src inhibitors; **SFK:** Src family kinases; **CAV-1:** Caveolin-1; **PcCa:** pancreas cancer; **HDAC(i):** histone deacetylase (inhibitor); **CTLc:** cutaneous T-cell lymphoma. **TRAIL-Ra:** tumor necrosis factor-related apoptosis-inducing ligand receptor agonist; **DR:** death receptor; **PARP-1i:** Poly (ADP-ribose) polymerase-1 inhibitor; **IGFR-1:** insulin growth factor receptor-1; **Hsp 90:** heat shock protein 90.

## **Discussion and Conclusion**

Today's intensive research is opening new windows, new perspectives, and delivering new options for BCa treatment. Far-off is the time when anti-estrogen therapy was the only effective therapy available and only for a subgroup of patients. Nowadays there is a wide range of therapeutic alternatives and many classes of different drugs. On the other hand, breast cancer is a very heterogeneous and complex disease, making the choice of treatment not usually an easy pick. Targeted therapies are generally very selective by nature, which renders them useless or with very low success rates if used indiscriminately. Furthermore, new targeted drugs are generally quite expensive, and represent a considerable "weight" in the already highly deficitary public health system. Thus, the development of biomarkers predictive of response to novel therapies is mandatory, in order to maximize treatment efficiency whenever possible. In this way, we can advance towards a true patient-tailored therapy, more adapted to each patient specific scenario.

Targeted therapies represent a new hope for advanced or metastatic BCa patients. Although most of the new drugs show very modest efficacy as single agents, there are very interesting synergic associations that worth further study like trastuzumab plus pertuzumab in HER-2 positive BCa, vorinostat and radiotherapy for treatment of brain metastases or even iniparib plus gemcitabine and carboplatin in triple negative BCa.

Even today, trastuzumab continues to have a fundamental role in the treatment of BCa patients, especially in those with HER-2 positive profile. Newer compounds like lapatinib and bevacizumab will probably be more frequently used in the future as more studies provide information on how to take full advantage of these innovative therapeutic agents. Hence, current and future research should focus on the critical task of answering important questions like: which drug or combination of drugs? in which patients? in which moment or sequence? and no less important, at what cost/benefit ratio? In this way, sustainable, intelligent, and more accurate treatment may, in time, become a reality.

## **Declaration of interest**

The authors declare that they have no potential conflicts of interest.

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## Appendix

### Abstract (em português)

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A neoplasia maligna da mama é uma das principais causas de morbidade e mortalidade por cancro. Nos estadios avançados de doença as opções terapêuticas encontram-se em franca expansão. A intensa investigação nesta área contribui decisivamente para o desenvolvimento de novos fármacos. O trastuzumab não é mais a única opção disponível nos doentes com cancro da mama receptores de estrogénio (ER) negativos, receptores de factor de crescimento epidérmico-2 (HER-2) positivo. Novos compostos anti-angiogénicos como o bevacizumab, assim como o inibidor das tirosina cinase do HER-1 e HER-2 estão actualmente aprovados pela FDA e pela EMEA para o tratamento no cancro da mama em doentes seleccionados. Outras terapias emergentes incluem os inibidores do alvo da rapamicina (mTORi), inibidores da farnesil transferase (FTi), inibidores da histona deacetilase (HDACi), inibidores da poli (ADP-ribose) polimerase-1, entre outros. Todavia, a selecção do alvo molecular, toxicidades associadas e a razão custo/benefício são questões que devemos ter presentes aquando da elaboração de um plano terapêutico. Esta revisão procura sumarizar a informação da literatura publicada sobre as terapias moleculares dirigidas e respectivos biomarcadores predictivos de resposta.

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FDA: Food and Drug Administration; EMEA: European Medicines Agency.