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Review

Treating Breast Cancer in the 21st Century: Emerging Biological Therapies

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Abstract

For many years, the medical treatment of breast cancer was reliant solely on cytotoxic chemotherapy. However, over the past twenty years, treatment has evolved to a more target-directed approach. We now employ tailored therapy based on the presence or absence of receptors for estrogen, progesterone, and human epidermal growth factor 2 (HER2). We expect this trend to continue, as agents that use novel approaches to target HER2, as well as targeting different portions of the HER signaling pathway, are in various stages of development. Notably, pertuzumab, a humanized monoclonal antibody that binds to a different domain of the extracellular portion of the HER2 receptor than trastuzumab, was recently approved for use, as was lapatinib, a small-molecule tyrosine kinase inhibitor. Patients with triple negative breast cancer, particularly those with the BRCA mutation, have more limited treatment options and carry a worse prognosis than those who are hormone receptor positive. However, recent data has shown that PARP inhibitors may have significant anti-tumor effect in those with this subtype of breast cancer. Novel agents that inhibit mTOR, PI3K, the insulin-like growth factor, heat shock protein 90, and histone deacetylase have shown promise in phase I-III trials and offer exciting new possibilities for the treatment of this often fatal disease. As we are presented with an ever increasing number of treatment options, the timing and combinations of therapeutic agents used becomes ever more complex in the age of personalized care, but we are hopeful that ultimately this will lead to improved patient outcomes.

Key words: breast cancer, chemotherapy, novel therapeutics, biologics, HER2, PARP inhibitors.

Introduction

Breast cancer is the most common cancer in women worldwide (1,2,3). In 2011, an estimated 230,000 women were diagnosed with breast cancer in the U.S. alone, with an estimated 40,000 deaths, making it the second most common cause of cancer related death in women (4). While only approximately 5% of all newly diagnosed breast cancer patients in the U.S. present with metastatic disease at the time of initial diagnosis, up to one third of patients with early stage disease will subsequently develop metastasis (3).

Over the past two decades, breast cancer mortality rates have declined by approximately 30%, with corresponding improvements in 5-year overall survival rates to 90% (4). Despite these advances, metastatic breast cancer (MBC) remains incurable, with an estimated 5-year overall survival rate of only 23% (5). However, more recent data from the SEER-Medicare database painted a more pessimistic picture, reporting median overall survival of Medicare patients with MBC to be only 22 months (6).

The biological underpinnings of breast cancer, and the major pathways involved in tumor progression and metastases, are still incompletely understood. Breast cancer traditionally has been classified into three different subtypes based on the presence or absence of three receptors found on cancer cells (7). Hormone receptor (HR) positive breast cancers express estrogen and/or progesterone receptors (ER/PR), and constitute approximately 60% of all breast cancer cases (8). The oncogene human epidermal growth factor receptor 2 (HER-2/neu) is over-expressed in approximately 20% of all breast cancer cases; while approximately 20% of breast cancer cases are negative for the expression of ER,PR, and HER-2/neu, also known as triple negative breast cancer (TNBC) (9,10).

More sophisticated genomic microarray analyses have also corroborated the presence of several distinct intrinsic molecular subtypes of breast cancer: luminal A, luminal B, basal, normal breast-like, and HER-2 like subsets (11,12). Subsequent to this initial research, the claudin low subtype was also recognized as yet another distinct molecular subtype. In about 70% of cases, the molecular breast cancer subtypes correlate with the expression of ER, PR, and HER-2 (13). Research is currently focusing on the clinical utility of molecular profiling that in the near future may replace traditional immunohistochemical staining, as initial evidence suggests that molecular profiling may be more accurate in predicting prognosis (13-14) .

Patients with hormone receptor positive tumors typically receive endocrine therapy (e.g. selective estrogen-receptor response modulators [SERM] and aromatase inhibitors [AI]) as one of many options of their treatment. Moreover, patients with HER-2/neu overexpressing tumors typically receive anti-HER/2 targeted therapy in combination with cytotoxic chemotherapeutic agents. Patients with triple negative breast cancer (TNBC) do not have these targeted treatment options, with cytotoxic chemotherapy being the primary modality (15, 16). In this article, we will review the most promising novel biologic agents under clinical development over the last 5 years in women with MBC. We will focus primarily on agents that target the HER-2 receptor family, poly ADP ribose polymerase (PARP) inhibitors, the mTOR pathway, insulin-like growth factor receptors, heat shock protein 90, and histone deacetylase (HDAC) inhibitors.

Novel therapies targeting the HER signaling pathway

The human epidermal growth factor receptor 2 (HER2) is a cell membrane tyrosine kinase receptor member of the epidermal growth factor receptor (EGFR) family (2, 17) that is over-expressed in approximately 15 - 25% of primary human breast cancers, and is associated with poor clinical outcomes and aggressive tumor progression (18- 21). The first commercially available HER 2 targeting agent was the monoclonal antibody trastuzumab (Herceptin®) (22, 23). The humanized monoclonal antibody trastuzumab binds to an extracellular segment of the HER2/neu receptor, leading to inhibition of the proliferation of human tumor cells that overexpress HER 2 (22). The exact mechanism of action is not fully understood. The use of trastuzumab has proven to improve survival for patients with breast cancer overexpressing HER 2; as adjuvant therapy for patients with early stage disease (24) and in combination with chemotherapy or as monotherapy for patients with metastatic disease (9, 25-36).

Antibodies and antibody conjugates

Trastuzumab established a new treatment paradigm for HER2-positive breast cancer. It is currently the standard first line treatment for patients with HER2-positive MBC in combination with chemotherapy or as a single agent (26, 30, 37-40). Lapatinib is a reversible small-molecule tyrosine kinase inhibitor that also targets HER2 by interfering with downstream signaling through the HER-2 pathway; it binds to the ATP-binding pocket of the EGFR/HER2 protein kinase domain, preventing self-phosphorylation and subsequent activation (41, 42). Lapatinib is currently approved as first-line therapy in combination with letrozole for patients with MBC who overexpress HER2 and are estrogen receptor (ER) positive (42- 44), as well as for the treatment of HER2- positive MBC in combination with capecitabine for patients who progressed on prior therapy including a taxane, an anthracycline, and trastuzumab (45, 46).

Despite the beneficial impact of HER-2 targeted therapy, numerous patients still develop recurrences or progression of the disease due to trastuzumab resistance (43). In patients with HER-2 amplified breast cancer, approximately 70% may have primary re-

sistance to trastuzumab (26, 47). In addition, an important number of patients who achieve initial response to the treatment tend to develop secondary trastuzumab resistance (47, 48). Nevertheless, the continuous use of trastuzumab has shown to be beneficial, even in patients who develop MBC after its use as adjuvant therapy or whose disease progresses while receiving this medication (20, 49-51).

The antibody–drug conjugate (ADC) trastuzumab-DM1 (Genentech) combines, via a specially designed linker, trastuzumab with a fungal toxin DM1 (Emtansine), a potent cytotoxic- microtubulin disruptive chemotherapeutic agent derivate of maytansine (52, 53). This allows trastuzumab to be used in the targeted delivery of the potent chemotherapy drug DM1 (2).

Using trastuzumab mainly as a carrier, this ADC was designed to target the delivery of DM1 into the HER2 overexpressing cells via endocytosis (53), wherein DM1 is subsequently detached from trasuzumab and released intracellularly, causing inhibition of microtubule assembly and posterior apoptosis (54-57), which leads to the recently described mitotic catastrophe (58). Trastuzumab-DM1 has been shown to have a mild dosage-related toxicity profile (59) (transient thrombocytopenia, elevated transaminases, fatigue, and anemia are the most common reactions), with low inter-individual variability in its pharmacokinetic parameters (60) and a maximal tolerated dose of 3.6 mg/kg every 3 weeks (61).

The efficacy of this ADC has been demonstrated in both *in vitro* and *in vivo* models of trastuzumab resistant breast cancer (53). More recently it has been proposed that trastuzumab-DM1 is able to circumvent the cross-resistance phenomenon observed with the use of lapatinib and trastuzumab (58). Preliminary efficacy data of a phase Ib/II study of trastuzumab-DM1given with Pertuzumab in HER2-positive, trastuzumab pre-treated patients showed partial responses (PR) among 23 patients (62).

In a recent phase II study involving patients with HER2-positive MBC who had progressed on earlier treatment with a HER2-directed agents plus chemotherapy (n=112), patients were schedule to receive trastuzumab-DM1 at a dose 3.6 mg/kg every 3 weeks. An overall response rate (ORR) of 25.9% was reported with a median PFS of 4.6 months. The median duration of response was not reached due to a low number of events (63). Early results from another phase II study comparing trastuzumab plus docetaxel to T-DM1 in first-line HER2-positive MBC indicated comparable response rates of 41 and 48%, respectively, without docetaxel related toxicities (64). A recent update indicated that PFS was significantly longer with T-DM1 versus trastuzumab/docetaxel (52, 65). Currently there are two prospective randomized phase 3 trials designed to evaluate the efficacy of T-DM1 in the management of MBC compared to the current standard of care. First, MARIANNE is a three arm trial that compared trastuzumab plus a taxane to T-DM1 combined with a placebo or pertuzumab (66). This study met enrollment goals in April 2012. The second trial is the TH3RESA trial, where TDM1will be compared to treatment of physician's choice as third line therapy in women previously having received taxanes, trastuzumab, and capecitabine/lapatinib, with or without prior anthracyclines (67).

Results of the EMILIA study, an open-label, randomized phase 3 trial comparing T-DM1 to capecitabine plus lapatinib (XL) as second line therapy in women with MBC previously treated with anthracyclines, taxanes, and trastuzumab, were recently reported (68). Patients who had received T-DM1 had significantly longer median progression free survival (9.6 vs 6.4 months, HR=0.650 p <0.0001), with a trend towards longer median overall survival time ([1-year : T-DM1 84.7% (80.76–88.55%) versus XL 77.0% (72.40–81.50%), 2-year: T-DM1 65.4% (58.65–72.15%) versus XL 47.5% (39.20–55.89%)]. The median overall survival was not reached in the T-DM1 arm and was 23.3 months in the capecitabine plus lapatinib arm. T-DM1 was beneficial for patients in different sub-groups, including those with visceral metastases and positive ER/PR status. T-DM1 was also well tolerated; the most common grade 3 adverse events for T-DM1: thrombocytopenia (12.9% vs 0.2%), increased AST (4.3% vs 0.8%), and increased ALT (2.9% vs 1.4%), and for XL: diarrhea (20.7% vs 1.6%), palmar plantar erythrodysesthesia (16.4% vs 0) and vomiting (4.5% vs 0.8%) (68).

Pertuzumab is a humanized monoclonal antibody that binds to the HER2 receptor, binding to a different domain of the extracellular portion of the HER2 receptor than trastuzumab, and blocks HER2-dimerization (43, 69). This agent has been actively investigated in combination with trastuzumab, aiming to explore the theoretical advantage of using two HER2 targeted agents (43, 70) for more complete blockade of the HER-2 signaling pathway (37). The phase III trial (CLEOPATRA) showed that the addition of pertuzumab to trastuzumab plus docetaxel, when used as first-line treatment for HER2-positive metastatic breast cancer, significantly prolonged median PFS by 6.1 months (HR, 0.62; 95% CI, 0.51-0.75; P<0.001), with no increase in cardiac toxicity (71). These data led to the approval by the FDA on June 8, 2012 of the compound as Perjeta® in combination with docetaxel and trastuzumab as first line therapy for

HER2+ MBC.

The trial that led to pertuzumab's approval was a double-blind, placebo controlled, multicenter, phase III trial that enrolled 808 patients with HER2-positive MBC for first-line therapy. The patients were randomly assigned into two groups: 406 to the placebo plus trastuzumab plus docetaxel group (control group) and 402 to pertuzumab plus trastuzumab plus docetaxel group (pertuzumab group). The primary end point was independently assessed progression-free survival, and secondary end points included overall survival and safety (71). The median progression-free survival was 12.4 months in the control group vs. 18.5 months in the pertuzumab group. The median investigator-assessed progression-free survival was 12.4 months in the control group vs.18.5 months in the pertuzumab group (HR, 0.65; 95% CI,0.54 -0.78; P<0.001) (71).

Data for OS are not yet mature; the interim analysis of overall survival was performed after 165 events had occurred (43% of the pre-specified total number of events for the final analysis) and is expected to be complete in 2013 (71). With the exception of cardiotoxicity, all other adverse events (of any grade), including diarrhea, rash, mucosal inflammation, febrile neutropenia, and dry skin, were reported more frequently in the pertuzumab group (71). Data so far available suggest that pertuzumab and trastuzumab in combination is more efficacious in the treatment of HER-2 positive breast cancer than the use of a single anti-HER 2 agent in both MBC and preoperatively, as neoadjuvant therapy (43).

The NeoSphere trial assessed the effect of pertuzumab and trastuzumab plus chemotherapy in treatment-naive women with locally advanced, inflammatory or early stage HER2-positive breast cancer (72).

The patients were assigned to one of four groups: group A received trastuzumab and docetaxel, group B received pertuzumab, trastuzumab and docetaxel, group C received pertuzumab and trastuzumab, and group D received pertuzumab and docetaxel.

Patients given pertuzumab and trastuzumab plus docetaxel (group B) had a significantly improved pathological complete response rate (49 of 107 patients; 45.8% [95% CI 36.1—55.7]) compared with those given trastuzumab plus docetaxel (group A) (31 of 107; 29.0% [20.6—38.5]; p=0.0141), without substantial differences in tolerability (72). The most common adverse events of grade 3 or higher was neutropenia (61 of 107 women in group A, 48 of 107 in group B, one of 108 in group C, and 52 of 94 in group D) and febrile neutropenia (eight, nine, none, and seven, respectively) (72).

Ongoing clinical trials with other HER2 targeted agents

MM-302 is a liposomal encapsulation of doxorubicin attached to antibodies that target HER2-overexpressing cancer cells, attempting to limit off target toxicity to non-malignant cells (73). Currently an ongoing phase I study is attempting to establish the safety and pharmacokinetics of this agent in patients with HER-2 positive MBC (74).

Ertumaxomab (Rexomun; Fresenius Biotech, Hamburg, DE) is a hybrid trifunctional monoclonal antibody that targets the CD3 antigen on T cells and the HER-2 expressed on the tumor cells (75).

This compound forms a HER-2-ertumaxomab-CD3 complex, leading to the aggregation and activation of T cells, macrophages, dendritic cells, and natural killer cells, which results in the phagocytosis and death of the tumor cells (75). A phase II study was terminated due to a change in development plan from the sponsor, not due to safety concerns (76). Further clinical studies are required to determine the clinical relevance of this medication.

MM-111 is a novel antibody fusion protein that targets the HER 2/HER 3 heterodimer, and blocks the ligand binding to HER 2-3 heterodimers, thereby inhibiting downstream signaling (77). The fusion protein is conformed by two human single-chain variable fragment (scFv) antibodies linked to a modified human serum albumin (78). An ongoing phase I/II trial is evaluating the safety and tolerability of MM-111 in patients with HER 2 MBC (77).

Table 1. Novel HER-2 monoclonal antibodies, drug conjugates, or antibody fusion proteins under clinical development in breast cancer.

Drug Name	Description	Phase	References
TDM-1	Trastuzumab-DM1 conjugate	I-III	53-55,58,60-64,66-68
MM-302	Nanotherapeutic encapsulation of doxorubicin with attached antibodies	I	73,74
Ertumaxomab	Murine monoclonal antibody with two antigen-recognition sites(CD3 & HER-2/neu)	I	75,76
Pertuzumab	Recombinant humanized monoclonal antibody targeting Subdomain II of (HER2)	II-III	69,71,72
MM-111	Novel antibody fusion protein that targets HER 2/HER 3 heterodimer	I-II	77,78

Small molecule HER/EGFR inhibitors other than lapatinib

Afatinib (BIBW 2992) is an irreversible small molecule inhibitor of the ErbB-receptor family, targeting the intracellular tyrosine kinase of the HER-2 molecule (79-81). In initial phase I studies, diarrhea, vomiting, nausea, fatigue and rash were the most common adverse events. Results of an open-label phase II study in advanced HER2-positive breast cancer patients after failure of trastuzumab were recently published, showing clinical benefit in 53% of the patients enrolled (79). Additional phase II studies showed acceptable tolerance and moderate activity of afatinib in ER-positive, HER2-negative breast cancer patients (82-84).

Neratinib (HKI-272) is a small molecule irreversible pan-HER receptor tyrosine kinase inhibitor (activity against HER1, HER2, and HER4) (85). It does not have activity against HER3, which is relevant because HER3 does not have an intracellular domain with tyrosine kinase activity. Early phase studies established the dose limiting toxicity to be diarrhea.

TAK-285 is a novel oral small-molecule dual HER-2/EGFR tyrosine kinase. The initial phase I study in primarily Japanese patients with advanced cancers demonstrated good tolerance of TAK-285, with elevation in aminotransferases and hyporexia being the most prominent grade 3 dose-limiting toxicities (86). In the U.S., a multicenter phase I study designed to determine the pharmacokinetics in a more diverse population of patients with advanced cancer was recently completed, and the results are forthcoming (87).

ARRY-380 is an orally active, small molecule, reversible-selective inhibitor of the HER-2 tyrosine kinase receptor with antitumor activity in HER2-positive breast cancer *in vitro* and *in vivo* (88). These findings led to a phase I trial which found ARRY-380 to be well tolerated, with rash and diarrhea as the most frequent adverse effects, along with promising signs of clinical activity, especially in pretreated patients with HER2+ MBC (89).

In an open-label, phase II study Burstein et al, showed the clinical activity of neratinib in patients with advanced HER2+ MBC with and without prior trastuzumab treatment: 16-week PFS was 78% for patients with no prior treatment with trastuzumab (n = 64) and 59% for those who had previously received trastuzumab (n = 63), with the median PFS 39.6 and 22.3 weeks, respectively. The most significant toxicities were diarrhea, vomiting, nausea, and fatigue (90). Neratinib has been included as one of the arms of the ongoing I-SPY 2 trial (91).

Anti HER-3 agents

MM-121 is a fully human monoclonal antibody that binds to HER3 and blocks the binding of its ligand, leading to inhibition of HER3 signaling. Schoeberl et al, provided data suggesting that MM-121 can be an effective therapeutic agent for cancers with ligand dependent HER3 activation (93). Currently Moyo et al. are conducting a phase II study, with MM-121 plus exemestane vs. exemestane alone in ER+ and/or PR + and HER2 negative MBC patients who have progressed on prior anti-estrogen therapy (94).

Table 2. Novel targeted anti-HER/EGFR therapies.

Drug Name	Description	Phase	References
Afatinib [BIBW 2992]	Orally active, small molecule irreversible pan-HER TKI	I-II	79-84
Neratinib	Orally available, irreversible TKI of HER-2	I-II-III	85,90-92
TAK-285	Novel, orally active, dual HER2/EGFR inhibitor	I	86,87
ARRY-380	Orally active, reversible and selective ErbB-2 inhibitor	I-II	88,89
MM-121	Fully human monoclonal antibody ErbB3 (Her3)	I-II	93,94

Table 3. Novel drugs targeting HER-3.

Drug Name	Description	Phase	References
MM-121	Fully human monoclonal antibody ErbB3 (Her3)	I-II	93,94

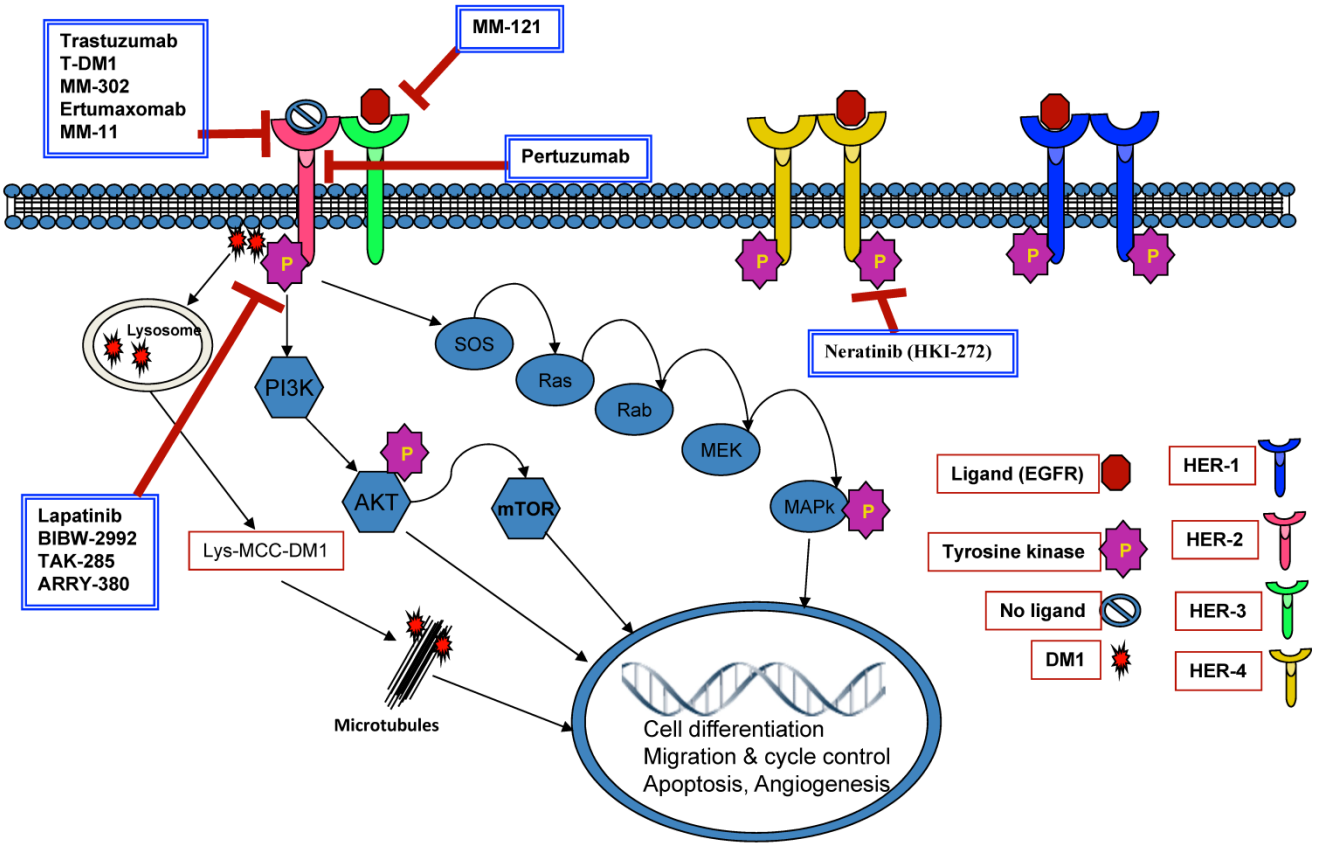

Figure 1. Therapies targeting HER signaling

Targeting DNA repair pathways: PARP1 Inhibitors

The poly (adenosine diphosphate [ADP])–ribose) polymerases (PARPs) are a family of enzymes involved in DNA repair, gene transcription, chromatin architecture, and apoptosis in normal human cells (95-97). The most abundant is PARP1, a key player in single-stranded DNA base-excision repair (95-100). PARP inhibition leads to the accumulation of single strand DNA breaks and subsequent double strand breaks at replication forks that ultimately induce apoptosis and cell death (100-102).

In normal cells, these breaks are repaired via the homologous recombination double-stranded DNA repair pathway. The tumor-suppressor proteins BRCA1 and BRCA2 are key components of the DNA repair pathway in humans (95,100). Normal cells, with intact homologous recombination, are able to tolerate the PARP inhibitors. However, in tumor cells lacking homologous recombination (i.e. BRCA1/2), PARP inhibition leads to cell death (101,102). This phenomenon has been described as synthetic lethality.

Farmer et al, demonstrated *in vitro* that PARP inhibitors are active only in the presence of BRCA mutations (103). The majority of woman with BRCA1 mutations develop triple negative breast cancer (102,103). This fact highlights the importance of the study of PARP inhibitors because currently there are no biological or targeted therapies approved for the treatment of BRCA-associated TNBC, which is an aggressive subtype of breast cancer with a poor prognosis after relapse (102). Iniparib, olaparib, and veliparib are the most investigated PARP inhibiting agents to date.

Until recently, iniparib was considered to be an irreversible inhibitor of PARP 1. However, Liu et al. showed that the primary mechanism of action of iniparib was not via the inhibition of PARP activity. Apparently, the cysteine-containing proteins in tumor cells are modified non-selectively by iniparib (104). O'Shaughnessy et al. conducted a phase II randomized study to compare the efficacy and safety of gemcitabine and carboplatin with or without iniparib. One hundred and twenty three patients with triple-negative MBC were included. The addition of iniparib prolonged the median progression-free sur-

vival from 3.6 months to 5.9 months (HR 0.59; P = 0.01) and the median overall survival from 7.7 months to 12.3 months (HR 0.57; P= 0.01), with no significant difference in the toxicity (neutropenia and thrombocytopenia being the most common grade 3 or 4 toxicities) between the two groups (105). Based on these results, a phase III trial was subsequently conducted in order to evaluate the overall survival and progression-free survival. Its primary endpoint was not met (106).

Olaparib (AZD2281- KU-0059436) is an orally active PARP inhibitor that induces apoptosis and cell death in homozygous BRCA-deficient cells (107). Fong et al. conducted a phase I study of olaparib focusing on BRCA1 and BRCA2 mutation carrying breast cancer patients, establishing the maximum tolerated dose and toxic profile of this agent. Olaparib was well tolerated, with grade 1-2 nausea, fatigue and vomiting being the most commonly observed adverse reactions (95). In the same study, investigators observed high rates of tumor response only in the BRCA1 or BRCA2 carriers (95). These promising results strongly suggest that BRCA mutation is necessary for the clinical activity of this agent in patients with MBC (100).

A phase II trial in patients with BRCA1 or BRCA2 mutation-associated, chemotherapy-refractory MBC evaluated two different dose schedules of olaparib: olaparib 400 mg once daily (cohort 1) vs. 100 mg twice daily (cohort 2). Overall radiographic response rates were 41% (11 of 27) for the first cohort and 22% (6 of 27). The most common grade 3-4 adverse reactions in both groups were nausea and fatigue (107). Gelmon et al. recently published results of a phase II study of olaparib in patients with advanced high-grade serous and/or undifferentiated ovarian carcinoma or TNBC. However, no confirmed objective responses were reported in the breast cancer group (108).

Veliparib is another potent PARP1 inhibitor (109). Donawho et al., using a breast xenograft model, were able to show the potentiating effect of veliparib when combined with cisplatin, carboplatin, and cyclophosphamide in tumor regression (110). Results of a phase I trial conducted by Kummar et al. established the maximum tolerated dose of veliparib at 10 mg orally twice a day, with neutropenia and thrombocytopenia as the most relevant dose limiting toxicities (111). A phase II study showed a synergistic activity for veliparib combined with temozolomide in breast cancer patients. The progression-free survival was 5.5 months in the BRCA-mutated group versus 1.8 months for patients without a BRCA mutation, findings that suggests that veliparib is only clinically active when the BRCA mutation is present (112).

Rucaparib (AG014699) demonstrates synergy with temozolamide in preclinical solid tumor models (113). A phase I trial of rucaparib combined with temozolomide in advanced solid tumors was conducted. This study established that doses up to 12 mg/m^2 AG-014699 and 200 mg/m^2 TMZ were safe and able to inhibit PARP in peripheral blood lymphocytes and tumor. No dose-limiting toxicity was observed. The adverse events were grade 1/2, except 1 case each of grade 3 infection, fatigue, low phosphate and lymphopenia, in a total of 27 patients. (114). Phase 2 trials are currently ongoing (115).

MK-4827 is an oral PARP-1/2 inhibitor. A recent study recruited 39 patients with multiple malignancies (11 of those were BRCA mutation carriers). PARP inhibition in peripheral blood mononuclear cells was observed at doses of > 110 mg daily, achieving antitumor activity in both BRCA mutated and not mutated tumors. Grade 3 fatigue, pneumonitis and anorexia were the most common dose limiting toxicity factors found (116). Currently a phase I study aims to determine the MTD and evaluate the degree of inhibition of PARP activity in patients with advanced solid tumors (including breast cancer) or hematologic malignancies (117).

Table 4. PARP inhibitors in clinical development.

Drug Name	Description	Phase	Target Population	Ref
Iniparib	Intravenous small-molecule prodrug inhibitor of PARP-1	I-III	triple-negative MBC	104-106,118
Olaparib [AZD2281]	Oral small molecule inhibitor of PARP	I-II	BRCA1 or BRCA2 mutation	95,107-108
Veliparib [ABT-888]	Oral PARP -1 and -2 inhibitor with chemosensitizing and antitumor activities	0-II	BRCA1 or BRCA2 mutation	109-112
Rucaparib [AG-014699]	Oral tricyclic indole PARP1 inhibitor with potential chemosensitizing, radiosensitizing, and antineoplastic activities	I	BRCA1 or BRCA2 mutation	113-115
MK-4827	Oral PARP 1/2 inhibitor	I	BRCA1 or BRCA2 mutation	116,117

Note: while only two trials (107,115) specifically examined BRCA-mutation associated malignancies, sub-set analyses of BRCA-mutation associated malignancies were done in the other trials listed above.

mTOR/PI3K

The mammalian target of rapamycin (mTOR) is a kinase that is part of the PI3K-related kinase family (119). mTOR plays a key role in cell cycle progression, and is inhibited by the antibiotic rapamycin (120). Over-activation of PI3K and mTOR has been observed in many cancers (121). As such, rapamycin, along with several rapamycin analogues (rapalogs), have been studied for the treatment of a variety of different cancers. The mTOR inhibitors temsirolimus and everolimus are currently approved for the treatment of renal cell carcinoma (122,123), and mTOR inhibitors have shown promise in a number of other types of cancers, including breast cancer.

Studies have shown that activation of the mTOR/PI3K pathway promotes anti-estrogen resistance (124). Pre-clinical models have shown that letrozole and everolimus both inhibit estrogen-induced breast cancer proliferation, and that these two agents in combination act synergistically to augment their anti-tumor activity even further (125). This cross-talk in activity has led researchers to combine an mTOR inhibitor and an aromatase inhibitor in the treatment of ER positive breast cancer. Everolimus has been the most widely studied mTOR inhibitor in breast cancer. The effect of everolimus added to an aromatase inhibitor was studied in a randomized phase II trial of 272 postmenopausal patients with operable ER positive breast cancer (126). Patients received 4 months of neoadjuvant letrozole combined with either everolimus or placebo. Response rate, determined by clinical palpation, favored the everolimus group 68.1% to 59.1%. In the everolimus group, 22.6% of the patients experienced a grade 3 or 4 adverse event, compared to 3.8% in the placebo group. A dose reduction or interruption in treatment due to an AE occurred in over half of the patients in the everolimus group. The most common grade 3 or 4 AEs were pneumonitis, pneumonia, and stomatitis. All three cases of pneumonitis resolved shortly after discontinuing everolimus.

Two schedules of everolimus as first or second line therapy in metastatic breast cancer were compared in a phase II trial of 49 patients (127). Patients were randomized to receive either 10mg daily or 70mg weekly doses of everolimus. Twelve percent of the patients in the daily therapy group responded, versus zero in the weekly therapy group. Pneumonitis occurred more frequently than expected; 11 of 33 patients in the daily group and 2 of 16 patients in the weekly group experienced grade two (symptomatic) or higher pneumonitis. Of note, most patients in this study had previously received radiation therapy. The most common adverse events include fatigue, rash, cytopenias, and elevated liver enzymes. Serious (grade 3 or 4) AEs include fatigue, infection, pneumonitis, cytopenias, and hyperglycemia, which lead to 27% of patients in the daily schedule and 13% on the weekly schedule to discontinue everolimus. Five of the 11 patients who withdrew from the study as a result of adverse toxicities did so due to pneumonitis.

BOLERO-2, a landmark phase 3 randomized placebo controlled trial, evaluated exemestane with or without everolimus in a 2:1 ratio in 724 postmenopausal patients with hormone receptor positive breast cancer who recurred or progressed while receiving a non-steroidal aromatase inhibitor (letrozole or anastrozole) and had advanced disease (128). Patients were allowed to have received other anticancer endocrine therapies and no more than one prior chemotherapy regimen. Patients were stratified, based on the presence of visceral metastasis and prior sensitivity to endocrine therapy, and received daily oral everolimus (10mg) or placebo combined with exemestane (25mg daily). Sensitivity to endocrine therapy was defined as at least 24 months before recurrence in patients receiving endocrine therapy as adjuvant therapy and 24 weeks of at least stable disease in patients with advanced breast cancer. PFS was the primary endpoint, with OS, ORR, and clinical benefit rate, among others, as secondary endpoints. Patients continued treatment until they developed disease progression, unacceptable toxicity, or withdrew from the study. 724 women at 189 centers in 24 countries were enrolled, with 485 receiving everolimus. The median age of the enrolled patients was 62, with 56% having visceral disease, and 76% with bony metastasis. 36% of patients had metastatic disease involving at least 3 sites, with lung and liver being the most commonly affected organs. All patients were ER positive and HER2 negative; 72% were PR positive. Every patient had received prior AI therapy with either letrozole or anastrozole, 48% had previously received tamoxifen, 16% had previously received fulvestrant, and 68% prior chemotherapy.

At the first formal interim analysis, which occurred after 359 events (approximately 60% of the PFS events), the median PFS was 6.9 months in patients receiving the combination of exemestane plus everolimus, versus 2.8 months for exemestane alone (HR for progression or death 0.43, 95% CI 0.35-0.54). Updated data was recently reported at the 2011 San Antonio Breast Cancer Symposium. After 457 events, the median PFS by central assessment favored the group that received the combination of exemestane/everolimus vs. exemestane alone (11.0 vs.4.1 months; HR 0.36, 95% CI 0.28-0.45) (129). ORR and

clinical benefit rate favored the everolimus-receiving group, 12.0% vs.1.3% and 50.5% vs.25.5%, respectively. 22.6% of the patients in the exemestane-only group died, compared to 17.3% in the everolimus-exemestane group. Serious adverse events attributed to the study treatment occurred in 11% of patients receiving everolimus vs. only 1% of patients in the placebo arm. Discontinuation of therapy as a result of AEs occurred more frequently in the everolimus arm, 19% to 4%. Grade 3 or 4 AEs that occurred more frequently in the everolimus plus exemestane arm included: stomatitis/mucositis (8% vs.1%), anemia (7%vs. 1%), hyperglycemia (5% vs. <1%), dyspnea (4% vs. 1%), fatigue (4% vs. 1%), and pneumonitis (3% vs. 0%). The drug Everolimus, a kinase inhibitor, was recently approved (July 20, 2012) by the FDA for the treatment of postmenopausal women with locally advanced, unresectable or metastatic hormone receptor-positive, HER2-negative breast cancer (advanced HR+ BC) in combination with exemestane after failure of treatment with letrozole or anastrozole.

Other therapeutic targets

Multiple factors lead to resistance to anti-HER2 agents, including increased signaling via upstream growth factor receptors, such as those in the EGFR and IGFR1 families, PTEN mutations, and changes in the HER2 receptor. However, the major mechanism of trastuzumab resistance appears to be increased activation/signaling of PI3K/Akt (130). Preclinical models have shown that the combination of trastuzumab with an mTOR inhibitor act synergistically to inhibit tumor proliferation, and the addition of trastuzumab to an mTOR inhibitor reduces the activity of the PI3K, MAPK, and HER3 signaling pathways (131). As such, several studies examining the addition of mTOR inhibitors to anti-HER2 therapy as a means of overcoming developed resistance have been undertaken. The combination of everolimus and trastuzumab, with or without chemotherapy, has been explored in several phase I and II clinical trials (132 -135). The ORR of this combination therapy was approximately 20% in all 4 trials. Toxicities were tolerable, with the most common grade 3-4 AE being neutropenia. BOLERO-1 is a randomized, double-blind phase III RCT that will compare trastuzumab and paclitaxel, with or without everolimus, as first line therapy in women with locally advanced or metastatic breast cancer (136). In BOLERO-3, a double-blind, randomized phase III RCT, the addition of everolimus to vinorelbine and trastuzumab is being compared to vinorelbine and trastuzumab alone in women with HER2 positive, locally advanced or metastatic breast cancer (137). Both of these trials are currently enrolling patients.

Much less data is available in MBC with the mTOR inhibitor temsirolimus. To date, there have been two reported trials involving temsirolimus, one phase II trial (138) and one phase I (139). Of the 31 patients in the phase II trial, none had an objective response to temsirolimus.

PI3K inhibitors

Phosphatidylinositol 3-kinases (PI3K) are a family of enzymes involved in multiple important cellular functions including proliferation, cell growth, differentiation, motility, and survival (140). Aberrant activation of PI3K has been implicated in different cancers. The gene that encodes the p110α catalytic subunit of PI3K (PIK3CA) is the most commonly mutated gene in breast cancer (141,142). PI3K promotes estrogen receptor activity, and mutations of PI3K can mediate resistance to endocrine therapy (143). Clinical trials with PI3K inhibitors in breast cancer patients are still in early phases of development. There have been 2 phase I/II trials reported involving SAR245408 or SAR245409, pan-inhibitors of PI3K (144,145). GDC-0941, a pan-inhibitor of PI3K, has been studied in two phase I trials (146,147). A third trial involving GDC-0941 in combination with fulvestrant is also currently ongoing (148).

Insulin-like growth factor inhibitors

Insulin-like growth factors I and II (IGF-I and IGF-II) play an integral role in the growth and development of somatic tissue, including bone and skeletal muscle (148). IGFs bind to IGF-receptors (IGF-R), helping regulate cellular functioning (149). Expression of both IGF and IGF-R increase during fetal development and in several types of cancers, including breast cancer (150), making the IGF-R a promising target for antibody directed therapy. An anticipated complication of therapy that targets IGF-R is hyperglycemia, as targets to the IGF-R may also bind to the insulin receptor (151). Aside from directly leading to the inhibition of tumor growth, IGF-R targeted therapy may also play a role in treating cancer by reversing resistance to endocrine, cytotoxic, or other therapies that may develop during the course of treatment.

Monoclonal antibodies targeting the IGF receptor have been the subject of many phase I and II clinical trials. One phase III trial involving figitumumab, a monoclonal antibody (MOAB) that targets/inhibits IGF-1R, combined with chemotherapy in the treatment of non-small cell lung cancer, has been reported (151). The study was terminated early due to lack of clinical benefit. Serious adverse events occurring

more often in the figitumumab arm included dehydration, hyperglycemia, and hemoptysis.

Five agents that target the IGF-R pathway have been or are being studied in the treatment of breast cancer. Ganitumab (AMG 479), figitumumab (CP-751,871), dalotuzumab (MK-0646, h7C10), and cixutumumab (IMCA12) are MOABs that target IGF-1R, while BMS-754807 is an IGF-1R/insulin receptor kinase inhibitor. The effect of ganitumab combined with hormonal therapy was studied in a randomized phase II double blind trial of 156 patients with ER and/or PR positive metastatic or locally advanced breast cancer who had received prior anti-estrogen therapy (152). In a 2:1 allotment, patients received either ganitumab or placebo, combined with exemestane or fulvestrant, per investigator's choice. The addition of ganitumab to exemestane or fulvestrant did not appear to improve median PFS or ORR. Grade 3-4 SAE occurred in 42% of patients receiving ganitumab, the most common being: hyperglycemia (6%), neutropenia (6%), thrombocytopenia (4%), asthenia (4%), and transaminitis (4%). A second trial involving ganitumab in breast cancer is currently ongoing (85).

In a randomized phase II trial, 205 patients with stage IIIB - IV, hormone receptor positive breast cancer received figitumumab plus exemestane or exemestane alone as first-line therapy (153). There was a non-significant trend toward improved median PFS with figitumumab. The most common grade 3-4 SAE associated with figitumumab included: transient hyperglycemia, the development of diabetes mellitus, weight loss, elevated GGT, and fatigue. Grade 3-4 hyperglycemia occurred in 12% of patients. Dalotuzumab (MK-0646, h7C10) will be combined with ridaforolimus, an mTOR inhibitor, in a phase II trial that will explore the efficacy and safety of these two agents in the treatment of ER positive breast cancer that progressed on aromatase inhibitors (154). There are currently three trials involving cixutumumab (IMCA12) in the treatment of breast cancer in various phases of development (155,156). One of these trials is studying the combination of cixutumumab and temsirolimus, an mTOR inhibitor (158). BMS-754807, the sole TKI that targets the IGF-R that has been studied in breast cancer, will be compared to BMS-754807 plus letrozole in a phase II trial involving patients with locally advanced or metastatic ER positive breast cancer (158).

Table 5. mTOR, PI3K, and Insulin-like growth factor inhibitors in clinical development.

Drug Name	Description	Phase	Target Population	References
Everolimus	Inhibits the mTORC1 protein	I-III	ER or PR positive (126, 128) HER2 positive (132-137)	126-128,132-137
Temsirolimus	Inhibits the mTORC1 protein	I-III	ER, PR, or HER2 positive (138) TNBC or HER2 positive (139)	138,139
BEZ235	Dual inhibitor of PI3K and mTOR	I	ER positive	158
GDC-0941	PI3K inhibitor		HER2 positive (146)	146,147
SAR245408	PI3K inhibitor	I/II	HER2 positive	144
SAR245408 or SAR245409	PI3K inhibitor	I/II	ER or PR positive	145
Ganitumab (AMG 479)	MOAB that targets IGF-1R	II	ER or PR positive	152
Figitumumab (CP-751,871)	MOAB that targets IGF-1R	II	ER or PR positive	153
Dalotuzumab (MK-0646, h7C10)	MOAB that targets IGF-1R	II	ER positive	154
Cixutumumab (IMCA12)	MOAB that targets IGF-1R	II	HER2 positive (155) ER or PR positive (156) ER positive (158)	155,156,158
BMS-754807	IGF-1R/insulin receptor kinase inhibitor	II	ER positive	158

Heat Shock Protein 90 (HSP 90)

HSP 90 is a molecular chaperone protein which assists in the folding and stabilization of proteins vital to cell survival (160). It assists in the stability and function of many proteins associated with cancer cell propagation, including estrogen receptors, HER2, EGFR, VEGFR, BCR-ABL, AKT, FLT3, MET, BRAF, and CRAF, among others, making it an ideal target for cancer treatment strategies. Breast cancers that express higher levels of HSP-90 are associated with a higher nuclear grade, larger tumors, increased lymph node involvement, increased expression of HER2 and ER, and more aggressive clinical features, e.g. de-

creased survival (161). Several different HSP-90 inhibitors have recently been studied in phase II trials. Tanespimycin (17-AAG) was combined with trastuzumab in 31 patients with advanced, HER-2 positive breast cancer who had progressed on trastuzumab (162).The ORR was 22%, median PFS was 6 months, and median OS was 17 months. There were no grade 4 AEs. Grade 3 AEs that occurred in more than 5% of the patients were transaminitis (10%), headache (7%), and fatigue (7%). Five patients withdrew from the study, one each for: fatigue, decreased ejection fracture (which also had occurred when she received trastuzumab previously), depression, transaminitis, and an atypical reaction to therapy (tremor plus unresponsiveness to verbal stimuli). Tanespimycin was added to trastuzumab in 29 patients with HER2 positive MBC who progressed after receiving trastuzumab (163). Of the 21 evaluated patients, five had a PR. The most common AEs were fatigue (39%), diarrhea (33%), dizziness (24%), and headache (19%). No grade 3 or 4 AEs occurred in more than 5% of the patients.

Retaspimycin (IPI-504) was combined with trastuzumab in 26 patients with advanced, HER-2 positive breast cancer who had progressed on trastuzumab (164). Among the 20 evaluable patients, there was 1 PR and SD in 14. The only grade 3-4 toxicities were grade 3 transaminits in a patient with liver metastasis, grade 3 vomiting, and grade 3 hypokalemia due to grade 1 diarrhea. Ganetespib, an HSP-90 inhibitor with broader activity then tanespimycin, was studied as single-agent therapy in an unselected cohort of patients with locally advanced or metastatic breast cancer (165). Data on 14 patients has been reported thus far. Of the 10 evaluable patients, there is 1 PR, 1 minor response, and 2 SD. Grade 3 abdominal pain and diarrhea requiring dose reduction and asymptomatic and reversible elevation in serum amylase occurred in one patient each.

Histone Deacetylase Inhibitors

The transcriptional factor hypoxia-inducible factor-1 alpha (HIF-1 α) regulates multiple cellular signaling pathways (166). HIF-1 α promotes angiogenesis by increasing the expression of VEGF, and HIF-1 α-induced over-expression of VEGF has been identified in various malignancies (165). Histone deacetylase inhibitors have been shown to indirectly (167) and directly (168) negatively regulate HIF-1 α, offering promising options for the treatment of tumors, which proliferate via stimulation from VEGF.

The use of valproic acid as an HDAC inhibitor was tested in a phase I/II clinical trial conducted by Munster et al (176). In this study, 44 patients with advanced solid malignancies were enrolled in the phase I portion, with 15 women with MBC enrolled into the cohort expansion. Breast cancer patients in the cohort expansion group received 120 mg/kg/day of valproic acid followed by FEC100 (epirubicin 100 mg/m2 with 5-fluorouracil 500 mg/m2 and cyclophosphamide 500 mg/m2). Partial responses were seen in 9 of 41 (22%) patients in the phase 1 portion. Objective responses were seen in 9 of 14 (64%) evaluable patients at the dose expansion cohort, after a median number of 6 cycles of therapy. The most common observed toxicities were valproic acid–associated somnolence and epirubicin-induced myelosuppression (169).

Three other HDAC inhibitors have been clinically evaluated to date in breast cancer patients: vorinostat, entinostat, and panobinostat. Initial studies used an HDAC inhibitor as an adjunct to AI therapy in patients who had progressed on an AI. Vorinostat was combined with tamoxifen in 19 patients with MBC who progressed on prior AI therapy (170). Significant toxicities included two venous thromboembolic events and grade 3 fatigue in 3 patients. The most common grade 2 toxicities included: fatigue, nausea/vomiting, anorexia, bleeding, and myelosuppression. Of the 17 patients evaluated for efficacy, 1 had a CR, 3 had PR, and 1 had SD for 12 months. A phase II trial involving 27 patients who were progressing on AI therapy received entinostat while continuing their same AI therapy (171). One patient had a PR and one had SD > 6 months. The most common grade 3-4 toxicities were: fatigue, dyspnea, diarrhea, and lethargy. A phase II trial of 114 patients with ER positive metastatic breast cancer progressing on an AI in which patients will be randomized, in a double-blind fashion, to continue their AI with or without the addition of entinosat, is currently enrolling patients (172). Panobinostat and capecitabine, with or without lapatinib, was studied in a phase I trial involving 15 patients (173). There was one DLT of grade IV thrombocytopenia, as well as 2 cases of grade 3 thrombocytopenia. Grade 3 anemia, dehydration, fatigue, peripheral edema, and hand-foot syndrome each occurred in one patient.

Table 6. HSP-90 and HDAC inhibitors in clinical development.

Drug Name	Description	Phase	Target Population	References
Tanespimycin (17-AAG)	HSP-90 inhibitor	II	HER2 positive	162, 163
Retaspimycin (IPI-504)	HSP-90 inhibitor	II	HER2 positive	164
Ganetespib	HSP-90 inhibitor	II	advanced or MBC	165
Vorinostat	HDAC inhibitors	II	ER positive	170
Entinostat	HDAC inhibitors	II	ER positive	171, 172
Panobinostat	HDAC inhibitors	I	advanced or MBC	173

Concluding Remarks

The paradigm for treating breast cancer has changed rather dramatically over the last decade. Several targeted therapies, e.g. trastuzumab and anti-estrogen therapies, have greatly improved patient outcomes. A much more detailed understanding of the underlying biology that drives malignant progression and metastases has yielded other novel targets including PI3K/mTOR and PARP, which are currently being tested clinically. One of the challenges, particularly in treating metastatic breast cancer, is that even with improved systemic therapies, the disease remains incurable. As more drugs that target specific pathways are developed, tumors develop means to evade these agents, particularly when there is redundancy in most biologic processes. The robustness, evolvability, modularity, redundancy, diversity, system control, tolerance, and plasticity are hallmarks of network pathways (174) which will further lead to difficulties of individual compounds to be successfully used against cancer growth for longer periods of time. One potential strategy is to combine multiple drugs that hit biologically important pathways at different places. Such a combinatorial strategy (175) will definitely increase efficacy, but we must choose wisely which combinations to pursue in future clinical trials. We believe that more thorough preclinical testing may help us make more informed decisions on which combinations should be brought forward into the clinic, especially when it is unlikely that we will be able to empirically test every possible combination. The next decade will hopefully bring new treatment paradigms that will continue to build on progress made over preceding two decades, and further improve clinical outcomes and survival rates for patients with breast cancer.

For HER2 positive MBC, given the availability of several HER2 targeted agents and the anticipated approval of newer agents as detailed above, in the future the clinician may be challenged as to how to sequence these therapies in clinical practice. An evidence-based approach should guide therapeutic decision making in an era where economic considerations are also paramount. Certainly, this question will be the subject of future studies. However, if one uses the paradigm of choosing highly efficacious therapies which have the least toxicities, this can be a guiding principle upon which treatments are chosen.

The negative reputation of HER2 positive metastatic breast cancer is changing with the approval of several new and well tolerated HER2 targeted treatments. In some aspects, this disease may be considered a "double-edged sword" due to the aggressive tumor biology, but at the same time beneficial due to the availability of a biomarker that can become a target for successful therapy. This in turn gives clinicians the availability of several less toxic targeted therapies which can drastically change the natural history of the disease. In fact it speaks to how far we have come in treating breast cancer as not just one disease, as our treatments will become "personalized" to specific subtypes of the disease. We can tailor our therapy to the presence of functional genes: molecular profiling will become much more used in the near future and more such targeted compounds may become reality. Much work is of course still needed to unfold the complex personalized networks of tumor proliferation and resistance mechanisms (176).

Competing Interests

The authors have declared that no competing interest exists.

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