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CANCER CHEMOPREVENTION (R AGARWAL, SECTION EDITOR)

Targeting HER2 Positive Breast Cancer with Chemopreventive Agents

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Abstract Human epidermal growth factor receptor 2-positive (HER2+) breast cancer is a subtype of breast cancer that is exhibited in approximately 20–30 % of breast cancer cases. The overexpression of HER2 is typically associated with a more aggressive disease and poor prognosis. Currently, the therapeutic drugs trastuzumab and lapatinib are the most commonly used to combat HER2+ breast cancer. However, tumors can develop resistance to these drugs. A better understanding of the mechanism of how HER2+ breast cancer works will help aid the development for new therapeutic approaches which more closely target the source of the signaling dysfunction. This review summarizes four major points in the context of HER2 overexpressing breast cancer (i) HER2 as a molecular target in breast cancer therapy, (ii) current treatment options as well as ongoing clinical studies, (iii) animal and cellular models for the study of HER2 overexpressing breast cancer, and (iv) future therapies and chemopreventive agents used to target HER2+ breast cancer.

Keywords HER2 · Breast cancer · Triterpenoids · Rexinoids · Vitamin D compounds · Vitamin E

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Introduction

Despite advances in the prevention and treatment of breast cancer, it still remains the most commonly diagnosed female malignancy [1]. Breast cancer is a heterogeneous disease and is typically classified based upon the expression of three different receptors, the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) [2]. HER2 amplification has been implicated in approximately 20 to 30 % of human breast cancer cases [3]. HER2, also known as ErbB2 or *neu*, is a 185-kDa transmembrane glycoprotein receptor tyrosine kinase. HER2 is part of the epidermal growth factor receptor (EGFR/ErbB/HER1) family of growth factor receptors. Four members of the ErbB family, HER1 (EGFR), HER2 (ErbB2/neu), HER3 (ErbB3), and HER4 (ErbB4) dimerize in various combinations to activate signaling pathways which regulate cell growth, survival, and differentiation [4, 5]. The overexpression of HER2 leads to enhanced and prolonged signaling of these pathways, ultimately initiating antiapoptotic and cell proliferation signals in breast cancer [4, 6].

The first line of treatment for breast cancer has long been radiation, surgery, and chemotherapy [7]. However, targeting HER2 and its pathways in treatment has improved the outcomes in breast cancer patients [8, 9]. Currently, there are several treatment options specific to HER2+ breast cancer such as the monoclonal antibodies, trastuzumab and pertuzumab, and the dual tyrosine kinase inhibitor, lapatinib [8, 10, 11]. Despite the more targeted therapies, resistance to these drugs has been a major challenge to effectively treat HER2+ breast cancer [12, 13]. With increased efforts, new approaches and chemopreventive strategies targeting HER2+ breast cancer have emerged. These studies have led to promising therapies in the preclinical and clinical stages of development.

There are various HER2+ breast cancer cell lines and animal models that are used to test existing therapies, novel therapeutic approaches, and chemopreventive agents. The two most common HER2 breast cancer cell lines utilized are BT-474 and SK-BR-3 cell lines [14]. These cell lines are commonly used in xenograft animal systems along with the transgenic mouse mammary tumor virus (MMTV)/neu HER2-overexpressing mouse model [15]. These tools will be invaluable in the accelerated screening and testing of new compounds for HER2 targeted therapies. Over the past 25 years, the HER2 receptor has become of utmost importance for the development of therapeutic approaches to a common breast cancer subtype. Despite development in the field, resistance has emerged to existing therapies. This has called for novel therapies and chemopreventive strategies to effectively combat HER2+ breast cancer.

HER Signaling

The HER receptors are composed of three general domains: the extracellular domain to which ligands bind, the transmembrane domain which anchors the receptor in the membrane, and the intracellular tyrosine kinase domain involved in signal transduction [16]. The HER2 receptor can homo- and heterodimerize with other members of the HER family such as HER1, HER3, and HER4 [17–20]. The structural conformation of HER2 is very different than that of other HER family members. HER2 is unable to bind ligands and is in a constitutively activated conformation in which the extracellular domain is capable of dimerization with other receptors [16, 21]. Since HER2 is capable of dimerization, even in the absence of ligand, this provides some insight as to why HER2 is the preferred dimerization partner for other HER receptors [16, 22, 23]. On the contrary, the HER3 receptor has unique characteristics in that it has little to no intrinsic kinase activity [24, 25]. Despite this, HER3 is still capable of ligand binding and heterodimerization, at which time downstream signaling can be activated by intrinsic kinase activity from its dimerization partner [26]. Upon dimerization, the intrinsic kinase activity is activated, and tyrosine residues within the cytoplasmic tails of the receptors are phosphorylated. This phosphorylation event initiates intracellular signaling cascades [7, 27, 28].

There are three dimers that regulate signaling in HER2 overexpressing breast cancer, HER1/HER2, HER2/HER2, and HER2/HER3. Thus, increased HER2 expression leads to increased dimerization and a deregulation of downstream signaling. The signaling cascades that these dimers activate are involved in a diverse range of cellular processes such as cell growth, proliferation, survival, differentiation, invasion, and angiogenesis [5, 9, 29, 30]. Two major pathways that regulate these cellular processes are the Ras/Raf/MEK/MAPK cascade as well as induction of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway which in turn activates the NF-kB pathway [5, 31]. The combination of these signaling cascades allows HER2+ breast cancer to continue to proliferate as well as evade apoptosis [32, 33].

These receptors and pathways have become primary targets for current treatments as well as new therapeutics. The reason HER receptors and signaling have become such crucial targets is due to the overexpression of HER2, the primary dimerization partner of the HER receptors, in a large percentage of breast cancers. The HER2 signaling output deregulates cell proliferation, differentiation, and survival, which is the driving force of the growth and metastases of breast cancer. The inhibition of these receptors and their signaling pathways provides a strong approach to the treatment of the HER2 subtype of breast cancer, which could increase patient survival and decrease the chance of relapse.

Approved HER2 Positive Breast Cancer Therapies and Ongoing Clinical Trials

Currently, there are several treatment options targeting the HER2 receptor or its associated signaling pathways, ultimately leading to longer survival and improved patient outcome. Approved HER2 treatment options include monoclonal antibodies, small molecule RTK inhibitors, and the combination of these drugs with other chemotherapeutic agents. Drugs that are FDA approved and in ongoing clinical trials for HER2+ breast cancer are summarized in Table 1.

Trastuzumab

Trastuzumab, a monoclonal antibody which targets the HER2 receptor, was developed by Ullrich, Shepard, and Fendly at Genentech who developed murine antibodies against ErbB2 [34]. One of the more promising antibodies, 4D5, showed anti-proliferative effects in breast tumor cell lines [35]. This 4D5 antibody was humanized to be tested clinically [36], leading to the 1998 approval of trastuzumab (Herceptin, Genentech) [9] and provided one of the first drugs for metastatic breast cancer [17, 37]. The antibody targets the extracellular domain IV of the HER2 receptor [38]. This inhibits dimerization to other HER family members, hence blocking signaling pathways associated with HER receptor activation.

Trastuzumab works on a variety of pathways and several mechanisms of action have been elucidated in vitro. Trastuzumab has been shown to downregulate cyclin D1 and increase p27 levels, leading to inhibition of Cdk2 and cell proliferation in HER2 overexpressing cell lines, BT-474 and SK-BR-3 [39]. Inhibition of HER3 phosphorylation, disrupting PI3K activation and Akt phosphorylation, has also been demonstrated with trastuzumab in vitro [39, 40]. Another mechanism of trastuzumab activity involves the inhibition of HER2 cleavage and the suppression of enhanced intracellular signaling through p95HER2 [41]. Despite preclinical work

Table 1 FDA approved clinical studies of HER2 inhibitors against breast cancer

Therapy	Trade name	Type	Target(s)	Indication	In combination with:	Ref(s)
Trastuzumab	Herceptin® (Genentech; 1998)	Humanized monoclonal antibody	HER2—binds extracellular domain IV	HER2+ early-stage breast cancer as a second- or third-line treatment	Chemotherapy	[44, 45]
Pertuzumab	Perjeta™ (Genentech; 2012)	Monoclonal antibody	HER2—binds extracellular domain II	HER2+ metastatic breast cancer in patients who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease	Trastuzumab, docetaxel	[54••, 56]
Trastuzumab emtansine (T-DM1)	Kadcyla® (Genentech; 2013)	Monoclonal antibody—drug conjugate	HER2—extracellular	Patients previously treated with anti-HER2 therapy trastuzumab and a taxane chemotherapy		[60, 65, 66•]
Lapatinib ditosylate	Tykerb® (GlaxoSmithKline; 2007)	Reversible tyrosine kinase inhibitor (TKI)	HER1, HER2—intracellular	HER2+ advanced-stage breast cancer in patients who have received prior treatment, including an anthracycline, a taxane, and trastuzumab	Capecitabine (Xeloda®; Roche), Letrozole (Novartis)	[70, 71, 80, 81]
Everolimus	Afinitor ® (Novartis; approved for advanced kidney cancer in 2009)	mTOR inhibitor	mTOR complex 1	Currently phase III for HER2+ trastuzumab-resistant breast cancer		[139••]
Afatinib (BIBW 2992)	Gilotrif® (Boehringer Ingelheim; approved for non-small cell lung cancer in 2013)	Irreversible TKI	HER1, HER2—intracellular	Currently phase III for HER2+ metastatic breast cancer		[86•]
Neratinib (PB-272)	Developed by Puma Biotechnology	Irreversible TKI	HER1, HER2, HER4—intracellular	Currently phase III HER2+ breast cancer and metastatic HER2+ breast cancer		[82, 85]

shown in cell and animal models, mechanisms of trastuzumab action in human patients are much less understood.

In primary breast tumors, treatment with trastuzumab showed high levels of apoptosis and a decrease in the levels of nuclear phosphorylated Akt [42]. Serum taken from patients treated with trastuzumab showed a decrease in HER2 extracellular domain concentrations, suggesting that trastuzumab inhibits cleavage of HER2 in a clinical setting [43]. Trastuzumab as an adjuvant to chemotherapy showed an increased latency to disease progression, a more effective and prolonged response to chemotherapy and improved overall survival compared to chemotherapy alone [17, 44, 45]. Currently, trastuzumab is the only approved adjuvant treatment for early-stage HER2+ breast cancer [31]. Trastuzumab is also used against metastatic HER2+ breast cancer alone [46] or in combination with paclitaxel [17, 47] or docetaxel [48, 49]. Despite the multimechanistic approach of trastuzumab and improved survival in patients, resistance has emerged and disease progression has become a clinical problem [13, 50].

Pertuzumab

Pertuzumab (Perjeta; Genentech), recently approved in 2012, is an antibody against the extracellular domain II of HER2 which disrupts dimerization of HER2 with other HER family members [51]. Pertuzumab is slightly different than trastuzumab in the fact that it targets domain II of HER2, while trastuzumab targets domain IV of the HER2 receptor [51]. It has been specifically shown to block dimerization with the ligand-bound form of HER3 [52]. Treatment with pertuzumab has shown to downregulate PI3K/Akt and MAPK pathways leading to antiproliferative effects in tumors [10, 51–53]. Early studies in vitro showed that pertuzumab in combination with trastuzumab resulted in decreased cell survival of HER2 breast cancer cells, partially due to apoptotic effects [10]. The combination treatment also enhanced antitumor activity in a xenograft model of HER2-overexpressing cells [53]. Currently, pertuzumab is approved in combination with trastuzumab and docetaxel [54••]. It is being evaluated for use with chemotherapy in trastuzumab-relapsed HER2 breast cancer patients [55–58]. There are also ongoing clinical trials to test pertuzumab in combination with a drug recently approved in early 2013, trastuzumab emtansine (T-DM1) [59].

Trastuzumab Emtansine

Trastuzumab emtansine (T-DM1; Genentech) is an antibody drug conjugate consisting of the antibody trastuzumab bound to the microtubule binder, mertansine [60, 61]. This drug provides two effects; the inhibition of HER2-mediated signaling, as well as the cytotoxicity provided by mertansine when it enters the cells, binds tubulin, and causes cell death [62•]. This drug conjugate retains the mechanisms of tumor inhibition of trastuzumab, while delivering a cytotoxic agent more specifically to the cancer-causing cells [63]. Early studies of T-DM1 in HER2+ models show effective antitumor activity in trastuzumab- or lapatinib-resistant cells and xenograft [60, 63]. Recent studies also show that T-DM1 can improve the targeting of the $CD44^{high}$ $CD24^{low}$ tumor-initiating cell subset [64••]. T-DM1 is approved for metastatic breast cancer in patients who have previously been treated with a trastuzumab and a taxane or in patients that show tumor recurrence [65, 66•].

Lapatinib

Lapatinib (Tykerb, GlaxoSmithKline) is a reversible dual tyrosine kinase inhibitor of both HER1 and HER2 [67, 68]. The drug was initially approved for advanced stage HER2-overexpressing breast cancer for use in combination with capecitabine for patients who experienced disease progression with prior therapy [9, 69, 70]. In 2010, lapatinib was approved for use in combination with letrozole specifically for postmenopausal women with HER2 and hormone receptor-positive breast cancer [71, 72]. Lapatinib is also used as a second line of defense to trastuzumab in patients that do not respond well to trastuzumab therapy [73].

Lapatinib binds specifically to the adenosine triphosphate (ATP)-binding pocket of the HER1 and HER2 kinase domains and prevents phosphorylation and receptor activation [68]. In turn, the activation of downstream signaling pathways such as the MAPK and PI3K/Akt pathways is inhibited in breast cancer cells, thus reducing tumor growth and proliferation [74–76]. Lapatinib also enhances the apoptotic effects of trastuzumab when used in combination in vitro [75, 77, 78].

This data has led to clinical studies to test the effects of lapatinib in combination with trastuzumab to treat advanced HER2+ breast cancer that has no responded to trastuzumab [75, 79]. The theory behind this combinatorial approach is due to the different mechanisms of action in how these drugs work. Trastuzumab is a monoclonal antibody, which prevents the HER2 receptor from dimerizing, and lapatinib is a tyrosine kinase inhibitor, which will prevent nascent signaling through the HER dimers. Another benefit is that, unlike trastuzumab, lapatinib is a small molecule; therefore, it is better suited to penetrate the central nervous system (CNS) and has the potential to better control an advanced, metastatic breast cancer [31].

There are several major phase III clinical trials involving lapatinib that are currently underway, TEACH (Tykerb Evaluation After Chemotherapy) and NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization) [80, 81]. These studies will pave the way for approved use of lapatinib and trastuzumab in combination as an alternative treatment option for HER2+ breast cancer patients.

Neratinib

Neratinib (PB-272; Puma Biotechnology) is a dual tyrosine kinase inhibitor of HER1, HER2, and HER4 under investigation for the treatment of early- and late-stage HER2+ breast cancer [82, 83]. Neratinib is an irreversible tyrosine kinase inhibitor, unlike the reversible nature of lapatinib; this drug will covalently bind to the ATP pocket of the receptor kinase [83]. Cell cycle arrest as well antiproliferative effects correlate with the decrease seen in signaling in the HER2 model systems upon treatment with neratinib [83]. Neratinib showed promising growth inhibitory results in trastuzumab resistant cell lines as well as in HER2+ xenograft in vivo [84•]. There are a variety of clinical studies underway to test the effects of neratinib as a first-line treatment as well as treatment for relapsed or trastuzumab-resistant HER2 breast cancer both as a monotherapy and in combination with a multitude of other cytotoxic agents [85].

Afatinib

Afatinib (BIBW 2992; Boehringer Ingelheim), like neratinib, is a small-molecule irreversible tyrosine kinase inhibitor of HER family members in HER2+ breast cancer [86•]. Afatinib has recently completed a phase II study showing the beneficial effects in HER2+ breast cancer that has escaped trastuzumab treatment [86•]. Afatinib has also recently been assessed in a phase I study in combination with trastuzumab for patients with advanced or metastatic HER2+ breast cancer; however, this study warranted further investigation due to inconclusive results [87].

Preclinical HER2 Experimental Models

The development of novel HER2+ breast cancer therapy is an arduous process with the end goal of the therapy reaching clinical trials and making it to the market. There is a long road of optimization to achieve effective target inhibition, while maintaining safety and reducing the potential for drug resistance. There is a vast array of established cell lines and animal models available for the assessment and optimization of potential HER2 therapies.

In vitro HER2 models Two of the most common HER2+ cell lines that are used in drug screening are BT-474 and SK-BR-3. These cell lines have been utilized in the development of current drugs such as lapatinib and trastuzumab [7, 14]. Although both cell lines show HER2 overexpression, the BT-474 line shows lower levels of the other HER receptors and the SK-BR-3 line shows an increased level of HER1 (EGFR) [88]. Other notable cell lines that have been tested and have shown resistance to trastuzumab treatment are MDA-MB-453, SUM-190, SUM-185, SUM-206, SUM-225, HCC1569, and JIMT1 cell lines [89–91]. These cell lines have a specific role for future research that could counteract current resistance to trastuzumab. Common HER2+ cell lines such as SK-BR-3, BT-474, and MDA-MB-361 have been conditioned with trastuzumab and lapatinib in a cell culture to create resistant cell lines to test new drug candidates that can overcome this resistance [63, 75].

In vivo HER2 models The most commonly used mouse model for the study of HER2+ breast cancer is the mouse mammary tumor virus (MMTV)-HER2/neu model (also known as MMTV-ErbB2 model) [92, 93]. This is a transgenic mouse model that overexpresses the active form of the rat oncogene, neu (the rat homologue of HER2) [92, 93]. The tumors that form in this model system resemble human ductal carcinoma in situ (DCIS) and are typically estrogen receptor negative and hormone independent [94, 95]. Since the MMTV-HER2/neu model represents a common subset of breast cancer, it has been extensively utilized for cancer treatment and prevention studies. Another commonly used transgenic model is the FVB/n Tg (MMTV-PyVmT) mouse. This model utilizes a HER2 mimic, which resembles the effect of HER2 heterodimers in a ligand- and dimerization-independent manner. This is an extremely efficient model showing 100 % penetrance, in which tumors form around 20 weeks of age. Lung metastases also appear in this model.

Experimental Therapeutic Approaches for HER2+ Breast Cancer

Despite advancements in HER2+ targeted therapies, resistance and disease progression have become clinical problems with current treatments, such as lapatinib and trastuzumab. Therefore, there is a variety of preclinical work investigating the effects of chemopreventive compounds on HER2+ breast cancer. Recent experimental drugs targeting HER2+ breast cancer are summarized in Table 2.

Triterpenoids

The development of new classes of drugs for the treatment and prevention of HER2+ breast cancer is critical, especially in the clinical setting where resistance is becoming an obstacle. A preclinical class of compounds, the synthetic triterpenoids, has shown promising therapeutic effects against HER2+ breast cancer [96]. Triterpenoids are plant derivatives of which more than 20,000 occur naturally [97]. Two specific triterpenoids which are antitumorigenic in vivo are oleanolic acid (OA) and ursolic acid (UA) [98]. These naturally occurring compounds have been synthetically optimized for many different functions to obtain a variety of synthetic oleanane triterpenoids with various functional groups [98–100]. The use of the

Table 2 Experimental therapeutic approaches for HER2+ breast cancer

Strategies and drugs	In vitro HER2 models tested	In vivo HER2 models tested	Treatment and/or prevention	Ref(s)
Triterpenoids				
CDDO	MCF7 (HER2 transfected); MDA-MB-435 cells (HER2 transfected)	MCF7 (HER2 transfected) cell xenograft	Prevention and treatment	[96]
CDDO-Me	E-18 cells	MMTV/neu transgenic mice	Prevention and treatment	[101, 138]
CDDO-Im		MMTV/neu transgenic mice	Prevention	[102•]
Rexinoids				
LG100268	E-18 cells	MMTV/neu transgenic mice	Prevention and treatment	[101, 138, 140]
LGD1069 (bexarotene)		MMTV/neu transgenic mice	Prevention	[106, 107, 141]
Vitamin D compounds				
BXL0124	E18-9A-42 cells	MMTV/neu transgenic mice	Prevention	[102•, 111]
1α(OH)D5	BT-474		Treatment	[112]
Vitamin E analogs				
α-TEA	MDA-MB-453		Prevention	[114]
α-TOS			Prevention	[115]
Resveratrol	SK-BR-3 cells; N2O2 murine cells; 4T1 cells	FVB/N HER2/neu	Prevention and treatment	[117, 118]
Genistein	BT-474; MDA-MB-435 cells (HER2 transfected); MCF7 (HER2 transfected)	MMTV/neu transgenic mice	Prevention	[120–123]

synthetic oleanane, 2-cyano-3, 12-dioxooleana-1, 9-dien-28-oic acid (CDDO), has been shown to inhibit cell cycle progression and reduce cyclin D1 levels through increased expression of the cyclin-dependent kinase inhibitor, p21 [96]. CDDO has also been shown to reduce tumor burden, HER2 phosphorylation, and cyclin D1 levels and induce apoptosis in xenograft with MCF-7 cells transfected with *ERBB2* [96]. Another synthetic oleanane triterpenoid, CDDO-Me, has been shown to inhibit proliferation and induce apoptosis in E-18 cells and also has reduced the onset of tumors in the mouse mammary tumor virus (MMTV)/neu, transgenic HER2+ mouse model [101]. Studies utilizing the synthetic oleanane triterpenoid, CDDO-Im, demonstrated a delay in mammary tumor onset in the MMTV/neu model [102•]. CDDO-Im decreased the activation of HER2 as well as HER1 and HER3 family members, thus repressing the activation of downstream signaling molecules, Erk1/2 and Akt [102•]. Triterpenoids show growth inhibitory effects in HER2+ breast cancer models, exhibiting the therapeutic potential of these compounds. Triterpenoids can be further explored as combination therapies with other agents or with currently established chemotherapies to fully utilize their anti-HER2 properties.

Rexinoids

Rexinoids are compounds which bind the three retinoid X receptors (RXRα, RXRβ, RXRγ) [103]. The RXRs are nuclear transcription factors that regulate a variety of cellular processes including cell proliferation, differentiation, and apoptosis [104•]. Due to their diverse regulatory potential, RXRs have become a popular target for drug design. One effective rexinoid, LG100268, has been shown to reduce proliferation and induce apoptosis in cells derived from a tumor extracted from a transgenic HER2+ mouse model [101]. LG100268 has shown to be an effective treatment strategy by exhibiting tumor regression of established MMTV/neu tumors in vivo [101]. LG100268 has also proved itself in chemoprevention by inhibiting tumorigenesis in a transgenic model of HER2+ breast cancer [105]. Another rexinoid, LGD1069 (bexarotene), prevented the formation of mammary tumors in a HER2+ mouse model [106]. Bexarotene has also showed promise in combination with a COX-2 inhibitor in a murine model of HER2+ breast cancer [107]. These studies of rexinoids in combination with other therapies show promise in the control of HER2+ breast cancer; however, more extensive combination studies are warranted to uncover the full potential of rexinoids.

Vitamin D Compounds

Vitamin D analogs have been used in the prevention of breast cancer in various animal models [108–110]. More specifically, the effects of vitamin D and its analogs are being investigated in HER2+ breast cancer models. Treatment with the Gemini vitamin D analog, BXL0124, in the MMTV-ErbB2 transgenic mouse model displayed a reduction in tumor burden [102•, 111]. BXL0124 downregulated HER2 phosphorylation without affecting the total HER2 levels in these mammary tumors. In addition, ERK and AKT phosphorylation were inhibited with BXL0124 treatment [111].

Another study investigated the potential of conjugating a vitamin D analog, 1α(OH)D5, and HER2-specific antibody to target HER2+ breast cancer [112]. Recent studies show that 25-hydroxy vitamin D supplementation in nonmetastatic HER2+ breast cancer patients was associated with improved disease-free survival [113]. With further confirmation of these studies, vitamin D compounds could hold the capacity to enhance the efficacy of current chemotherapies and HER2+ breast cancer treatments.

Vitamin E Analogs

Studies have shown that the dietary intake of vitamin E has reduced the risk of developing breast cancer. Recent studies with alpha-tocopheryloxyacetic acid (α-TEA), a novel ether derivative of alpha-tocopherol, suppressed mammary tumor growth and reduced the incidence of lung metastases both in a transplanted and a spontaneous mouse model of breast cancer [114]. Another group showed that α-tocopheryl succinate (α-TOS) was capable of inducing apoptosis in HER2 low and HER2+ cells, suggesting that α-TOS could potentially overcome the pro-survival effects of HER2+ breast cancers [115].

Resveratrol

Resveratrol is a natural polyphenolic compound and has been widely studied for its anticancer properties [116, 117]. One study reported a delayed onset of tumors and reduced metastasis in a murine model of HER2 breast cancer treated with resveratrol [118]. This study also showed that resveratrol reduced HER2 gene expression and induced apoptosis in human and murine cells that overexpress HER2 [118]. Despite numerous studies as a breast cancer chemopreventive compound, resveratrol has not been extensively studied in HER2+ breast cancer and warrants more thorough studies to confirm its effects.

Genistein

Genistein is the main isoflavone found in soy and has shown to be a potent tyrosine kinase inhibitor [119]. Therefore, it has been studied rather extensively with respect to HER+ breast cancers [120, 121]. MMTV-HER2/neu mice that fed on diets containing genistein demonstrated a significant delay in mammary tumorigenesis [122]. Genistein demonstrated a downregulation of HER2 protein expression and phosphorylation in HER2-overexpressing human breast cancer cells [123]. The photoactivation of psoralen with UVA irradiation, referred to as PUVA, reduced $p85^{ErbB2}$ phosphorylation leading to tumor cell apoptosis [124]. The use of genistein, flavonoids, or soy products may be potentially beneficial in the prevention of HER2+ breast cancer.

HER2+ Breast Cancer Resistance

Despite the advancements in therapy for HER2+ breast cancer, trastuzumab resistance has emerged [125]. Since trastuzumab works to inhibit HER2 signaling through various mechanisms, it has been difficult to pinpoint how HER2+ breast cancers develop resistance to this treatment. Several modes of resistance have been elucidated in vitro; however, only some of these have been confirmed in a clinical setting.

A well-studied mechanism for resistance to trastuzumab is the shedding of the extracellular domain of the HER2 receptor, generating a constitutively activated intracellular fragment, known as p95HER2 [126, 127]. This is unable to bind trastuzumab but is still capable of initiating signaling cascades. The trastuzumab-resistant p95HER2 fragment still responds to treatment with lapatinib in vitro since lapatinib specifically targets the kinase activity [127]. This mechanism has been linked to patients with HER2+ metastatic breast cancer in which p95 expression was associated with trastuzumab resistance [127]. Another mechanism of resistance suggests that the HER2-trastuzumab interaction is reduced by the increased expression of the membrane-associated glycoprotein, mucin 4, which competes with trastuzumab for the binding of HER2 [128, 129]. Another mode of resistance is to enhance signaling to evade treatment through the upregulation of the HER3 [130] and insulin-like growth factor 1 receptors (IGF-1R) [125, 131, 132]. Crosstalk between these receptors, as well as other HER family members, can enhance signaling to compensate for signaling lost from trastuzumab treatment [133]. An overexpression of HER family ligands is known to be involved in resistance due to increased receptor dimerization and activation [134]. Downregulation of p27 is known to confer resistance to trastuzumab treatment in vitro [135]. Constitutive activation of PI3K drives cell growth and proliferation and has been implicated as a major mechanism of resistance to trastuzumab [136]. This can occur through decreased levels of PTEN (phosphatase and tensin homologue deleted on chromosome 10) expression, which shows the necessity of PTEN in the mechanism of action of the drug in a clinical setting [137]. Patients with lower PTEN levels showed a decreased response to trastuzumab compared to tumors, which were PTEN-positive [137].

In order to develop more targeted therapies to avoid trastuzumab resistance, there is a need for a better understanding of how these pathways work, especially in the clinical realm. For this reason, the area of HER2-targeted therapies and chemoprevention still remains a vital area of breast cancer research.

Future Therapies

There are many new agents on the horizon, which include new and modified antibodies, tyrosine kinase

inhibitors, vaccines, as well as natural product derivatives that target the HER2 receptor and its associated pathways [31, 138]. Although HER2 is the direct target of many of these new treatment options, other therapeutics that are being developed which indirectly target HER2 signaling by focusing on other molecules involved in the process, such as mTOR, IGF-1R, HSP90, and VEGF, are also under investigation [31]. These new treatment strategies, along with existing regiments, will help to overcome the resistance associated with trastuzumab treatment. Many of these new drugs are being clinically evaluated in a combination approach or alongside currently approved therapies.

Conclusions

HER2, a common subtype of breast cancer, is overexpressed in approximately 20–30 % of cases, making it a significant therapeutic target for breast cancer. The family of HER receptors controls antiapoptotic pathways as well as proliferation mechanisms through the MAPK and PI3K/Akt pathways. Overexpression of the HER2 receptor allows for the upregulation of these pathways leading to breast cancer. The breadth of knowledge in the area of HER2 signaling has expanded greatly since its first implication in breast cancer nearly three decades ago. Despite advances in HER2+ breast cancer therapy, resistance to commonly utilized drugs has emerged. To combat this, there is a vast array of new drugs and combination therapies being evaluated clinically and preclinically. Among new treatments, various dietary phytochemicals have shown promise for the prevention and therapy of HER2+ breast cancer. An increased understanding of HER2 signaling pathways as well as novel in vivo and in vitro models to study resistance will provide a greater insight into the mechanisms of disease in HER2+ breast cancer. This ongoing research is vital to identify new therapeutic targets and develop chemopreventive agents to better target HER2+ breast cancer.

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Compliance with Ethics Guidelines

Conflict of Interest Joseph Wahler and Nanjoo Suh declare that they have no conflict of interest.

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- • Of importance
- •• Of major importance

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