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# Early clinical development of epidermal growth factor receptor targeted therapy in breast cancer

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## Abstract

**Introduction—**Epidermal growth factor receptor (EGFR) targeted treatment has been evaluated but has not shown a clear clinical benefit for breast cancer. This review article aims to consider the knowledge of the biological background of EGFR pathways in dissecting clinical studies of EGFR targeted treatment in breast cancer.

**Areas covered—**This review focuses on the role of the EGFR pathway and the investigational drugs that target EGFR for breast cancer.

**Expert opinion—**Recent studies have indicated that EGFR targeted therapy for breast cancer has some promising effects for patients with triple-negative breast cancer, basal-like breast cancer, and inflammatory breast cancer. However, predictive and prognostic biomarkers for EGFR targeted therapy have not been identified. The overexpression or amplification of EGFR itself may not be the true factor of induction of the canonical pathway as an oncogenic driver of breast cancer. Instead, downstream, non-canonical pathways related to EGFR may contribute to some aspects of the biological behavior of breast cancer; therefore, the blockade of the receptor could result in sufficient suppression of downstream pathways to inhibit the aggressive behavior of breast cancer. Mechanistic studies to investigate the dynamic interaction between the EGFR pathway and non-canonical pathways are warranted.

### Keywords

Breast cancer; EGFR; monoclonal antibodies (mAbs); tyrosine kinase inhibitors (TKIs); cetuximab; panitumumab; gefitinib; neratinib

## 1. Introduction

Epidermal growth factor receptor (EGFR, also called ErbB1 or HER1), one of the four members of the transmembrane EGFR family, plays an important role in signal transduction

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pathways that regulate cellular function, including cell survival, proliferation, angiogenesis, and inhibition of apoptosis [1]. At present, two categories of drugs have been identified to target EGFR: monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs). EGFR-targeted treatment is a standard of care in non–small-cell lung cancer (NSCLC) and colorectal cancer for selected patient populations. In breast cancer, EGFR-targeted TKIs, including gefitinib, erlotinib, and cetuximab, have been evaluated in clinical trials but have not shown a clear clinical benefit [2–5].

Successful targeted treatment depends on the selection of appropriate patients based on predictive biomarkers or biological understanding of how the target modulates disease aggressiveness. Thus, this review article aims to consider the knowledge of the biological functions of the EGFR pathway in dissecting the clinical studies of EGFR-targeted treatment thus far performed in breast cancer. In particular, we focus on current knowledge regarding anti-EGFR therapeutics. By doing so, we aim to build a framework for the use of these therapeutics in the setting of breast cancer, where anti-EGFR therapy is less established than in other solid tumors, e.g. lung and head and neck cancers.

## 2. Role of the EGFR pathway in the progression of breast cancer

### 2.1. Overview of the ErbB family of receptors and its major signaling pathways

Epidermal growth factor (EGF) was first discovered in the early 1960s in the submandibular gland of mice and shown to be a polypeptide that stimulates the cell growth and differentiation of epidermal- and mesodermal-originating cells [6]. In subsequent studies, the receptor of EGF (EGFR) and the intrinsic kinase activity of EGFR were identified [7]. EGFR is an integral membrane glycoprotein with a molecular weight of ~170 kDa. It was the first receptor that was shown to result in phosphorylation of tyrosine residues when bound by ligand. In 1984, Ullrich et al. cloned the complete amino acid sequence derived from human EGFR cDNAs, and Downward et al. identified several EGF-stimulated phosphorylation sites on EGFR [8,9]. It was also found that ligand binding induced receptor clustering and that antibody cross-linking mimicked the effects of EGF, indicating the importance of receptor dimerization/oligomerization in its activation [10]. These findings contributed to identification of other members of the ErbB family of receptor tyrosine kinases (RTKs): ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These RTKs regulate several signaling networks involved in cell proliferation, angiogenesis, survival, and metastasis [11,12].

The structure of EGFR consists of an extracellular domain, which has four segments (I = ligand-binding domain 1; II = cysteine-rich domain 1; III = ligand-binding domain 2; and IV = cysteine-rich domain 2), and an intracellular domain, which contains a juxtamembrane domain, a tyrosine kinase domain, and a carboxyl terminus [13]. The binding of ligand induces the dimerization of receptor and the subsequent activation of the catalytic domain of EGFR family members [14]. Dimerization is mediated by EGF's 'dimerization arm,' which contacts two distinct sites within a single receptor molecule (on domains I and III). This bivalent ligand binding promotes allosteric domain rearrangement that results in substantial conformational changes in the extracellular region of EGFR and subsequent transphosphorylation of the tyrosine residues located in the cytoplasmic tail [15]. Another

mechanism of EGFR activation upon ligand stimulation is the asymmetric dimerization of the kinase domains of EGFR, in which the carboxy lobe of the 'activator' kinase in an EGFR tyrosine kinase domain dimer is thought to induce allosteric changes in the amino lobe of the 'receiver' kinase and thus activate it [16]. This asymmetric dimerization also activates EGFRvIII, an oncogenic mutant form of EGFR [17].

EGFR forms heterodimers with other ErbB family members, which generate more potent signals than EGFR homodimers because heterodimerization provides additional phosphotyrosine residues for the recruitment of effector proteins, which induces distinct patterns of receptor phosphorylation and downstream signaling, as well as reduces receptor internalization and degradation as compared to homodimerization [18]. The heterodimerization of EGFR with other family members also diversifies the signals [19–21]. EGFR also forms heterodimers with other RTKs, such as platelet-derived growth factor β receptor (PDGFβR), insulin-like growth factor 1 receptor (IGF-1R), and c-Met. Platelet-derived growth factor stimulation can induce the transactivation of EGFR upon reactive oxygen species production and Src activation and the formation of an EGFR– PDGFβR heterodimer [22]. Treatment of NSCLC cells with EGFR TKIs induced the formation of an EGFR–IGF-1R heterodimer and the activation of IGF-1 R and downstream signaling, with the cells subsequently developing resistance to treatment with the TKI erlotinib [23]. The heterodimerization of EGFR with c-Met results in the EGFR-dependent activation of c-Met in the absence of its ligand, hepatocyte growth factor, and has been demonstrated to be related to resistance to EGFR-targeted drugs [24].

Several downstream pathways – such as phosphoinositol-3-kinase (PI3K)/AKT, RAS/RAF/MEK/ERK, STAT, and phospholipase c (PLC)/protein kinase-C (PKC) – mediate the signals of ErbB receptor activation to induce epithelial cell migration and invasion in cancer (Figure 1) [25–32]. Several of these pathways are interrelated. The PI3K/AKT serine/threonine kinase 1 (AKT) pathway initiates AKT phosphorylation and activation by mediation of phosphoinositide-dependent kinase 1 and mammalian target of rapamycin complex 2 [25,26]. Activation of AKT also activates the translocation of nuclear factor kappa B (NF-κB) from cytoplasm to nucleus and induces the expression of NF-κB-targeted genes that are involved in cancer invasion and metastasis [27,28]. Simultaneously, AKT is attenuated by phosphatase and tensin homologue deleted in chromosome 10 [29]. The ERK pathway also plays a role in tumor cell proliferation and survival. Activated EGFR promotes the guanosine triphosphate (GTP)-binding RAS, which is recruited by guanine nucleotide exchange factors that phosphorylate MEK1 and MEK2 [30]. Phosphorylated MEK1 and MEK2 mediate the ERK pathway and subsequently activate a number of transcription factors. In addition, PI3K/AKT and signal transducer and activator of transcription (STAT) are activated by EGFR pathways [31]. Another downstream pathway of EGFR signaling, PLC/PKC, is initiated when PLC-γ binds to phosphorylated EGFR tyrosine kinase to become active. Once activated, PLC-γ hydrolyzes phosphatidylinositol 4,5-biphosphate to diacylglycerol (DAG) and inositol triphosphate. DAG is a cofactor for the activation of the serine/threonine kinase PKC, and the activation of PKC regulates cell cycle progression, transformation, differentiation, and apoptosis [32].

Suppression of EGFR signaling has shown efficacy in controlling the progression of breast cancers, with three mechanisms thought to be involved. First, in preclinical work, EGFR suppression was shown to suppress the stem cell population in breast cancer [33,34]. Second, suppression of the EGFR pathway enhances apoptosis in cancer cells via the downstream PI3K/AKT and PLC/PKC pathways [9,19–21]. Third, the EGFR pathway may regulate epithelial–mesenchymal transition (EMT) in breast cancer cells [34–37]; this mechanism is discussed further in the next section.

### 2.2. Role of EGFR in regulation of EMT

EMT is the process of acquisition of molecular alterations by which epithelial cancer cells lose their epithelial features (E-cadherin and cytokeratin) and gain mesenchymal features (vimentin, N-cadherin, Snail, Slug, and Twist) [38,39]. ErbB family members initiate EMT directly and/or indirectly and are involved in cancer progression and metastasis [37]. This process has been shown to govern distant metastasis in epithelial cancers, including breast cancer. The transcription factors Snail, Slug, Twist, and zinc-finger E-box-binding homeobox 1 and 2 (ZEB1 and ZEB2) are classified as EMT inducers [40,41]. These transcription factors are inappropriately activated in cancer and are believed to contribute to tumor progression via different cell signaling pathways. Preclinical and clinical studies have demonstrated that EMT markers are associated with limited response to EGFR treatment [35,36]. EMT may underlie development of EGFR TKI resistance in NSCLC. In breast cancer, a preclinical study by Zhang et al. suggested that suppression of EGFR reduces the expression of EMT markers that are known to increase the metastatic potential of breast cancer cells [34]. Lo et al. demonstrated that EGF exposure induces EMT in breast cancer cells, and this phenotypic transition regulates EGFR-mediated activation of STAT3 via STAT3-activated *TWIST* gene expression [42]. The cross talk between EGFR and other signaling pathways involved in EMT, including transforming growth factor (TGF)-β, Notch (a family of transmembrane proteins), and Wnt (wingless-type MMTV [mouse mammary tumor virus]), has been implicated in inducing EMT in epithelial cells, including breast cancer cells [43,44]. Taken together, these findings demonstrate that EGFR signaling has an important role in both cancer progression and EMT-associated invasion.

### 2.3. Tumor microenvironment and EGFR signaling

A tumor’s microenvironment has been recognized as an important contributor to tumorigenesis and metastasis. Recent developments in immuno-oncology have widened the area of research focusing on the components of the tumor microenvironment. Tumor cells need sufficient support and supply of blood, oxygen, and nutrients from the surrounding area, such as stromal cells, fibroblasts, and immune infiltrates [45]. The homeostasis between immune-related cells and tumor cells has been recognized as a key mechanism of the balance of tumorigenesis vs. apoptosis that ultimately contributes to the character and behavior of cancer for each patient.

Mesenchymal stem cells (MSCs) are multipotent progenitor cells found in normal tissues that can differentiate into a variety of cell types [46]. In a xenograft model, co-injection of MSCs with SUM149 inflammatory breast cancer cells increased skin invasion and metastasis in mice, mimicking clinical features of inflammatory breast cancer (IBC)

(presenting as erythema and inflammation) compared with injection of SUM149 cells alone [47]. Compared with mice in which MSCs were not co-injected, primary tumors in mice with injected MSCs expressed higher phosphorylated EGFR levels and were associated with increased metastasis development after tumor resection, effects that were abrogated by treatment with the EGFR inhibitor erlotinib. These results suggest that the tumor microenvironment promotes the IBC clinical phenotype and that the EGFR pathway mediates the cross talk between IBC tumors and the microenvironment.

Macrophages are directly involved in tumor progression and metastasis by facilitating angiogenesis and extracellular matrix breakdown and remodeling and promoting tumor cell motility [48]. EGFR signaling plays a role in a paracrine loop of macrophages with tumor cells during the initial stages of metastasis. In this loop, macrophages associated with the vessels secrete EGF, which stimulates cancer cells through EGFR; cancer cells secrete colony stimulating factor 1 (CSF-1), which attracts macrophages and induces them to express EGF [48].

EGFR has been shown to have a connection with the inflammatory pathway, especially the COX2 pathway (unpublished data). COX2 is an inducible isozyme that catalyzes the conversion of arachidonic acid to prostaglandins and other prostanoids, which are involved in various aspects of the inflammatory response. COX2 is elevated in a number of malignancies, and its expression is associated with increased cancer cell growth, increased invasiveness, and poor prognosis in patients with breast cancer [49,50]. Increased levels of prostaglandins have been found in different types of human tumors, including breast tumors, and could be used as a marker of high metastatic potential in breast cancer [51,52].

### 2.4. EGFR gene amplification and mutation

Several studies have reported that *EGFR* gene amplification in breast cancer might be a predictor of response to TKIs. Reis-Filho et al. demonstrated that metaplastic breast carcinoma with basal-like differentiation frequently overexpressed EGFR, which can be associated with gene amplification [53]. On the other hand, it was identified that *EGFR* gene amplification was a rare event. One study reported an *EGFR* gene amplification rate of 6.3% of 95 cases of invasive ductal carcinoma; amplification was associated with shorter recurrence-free survival [54]. Sassen et al. reported that *EGFR* gene amplification was rare among EGFR-positive cases, and the combination of EGFR and HER2 expression was not associated with disease outcome [55].

Rapidly evolving technology has made it possible to analyze genomic characteristics of patients' samples using next-generation sequencing in a relatively short period of time. *EGFR* mutations have been detected in various cancers, and genomic activation has been shown to lead to aberrant and constitutive tumor progression. Activating somatic mutations in the *EGFR* gene conferring sensitivity to EGFR TKIs were first reported in 2004 in NSCLC. Since then, many Phase III clinical trials have established EGFR TKI therapy as a first-line treatment for NSCLC patients who have *EGFR* mutation. Recently, an additional class of EGFR oncogenic mutation, C-terminal deletion (CTED) mutants resulting from the combination of exonic deletions of exons 25 and 28, was identified through large-scale genomic studies in glioblastoma and lung cancer [56]. These mutants lead to the oncogenic

activation of EGFR through an asymmetric dimerization-dependent activation mechanism and are sensitive to EGFR inhibitors, suggesting that the CTED status of EGFR can be considered a potential genomic marker for EGFR-targeted therapy.

In breast cancer, many efforts have sought to discover targetable somatic mutations. The extensive genomic and proteomic analysis in 825 patients with breast cancer reported by The Cancer Genome Atlas showed that only three somatic mutations (*TP53*, 36%; *PIK3CA*, 37%; and *GATA3*, 11%) were detected at >10% incidence in all breast cancers [57]. As shown in this report, many of the components of the PI3K and RAS/RAF/MEK pathway, such as *PIK3CA* (49%), *KRAS* (32%), *BRAF* (30%), and *EGFR* (23%), were amplified in basal-like breast cancers (80% of which are triple-negative breast cancer [TNBC]). In addition, a previous report suggested that the majority of missense mutations of *EGFR* exist in its tyrosine kinase domain, and the rate of *EGFR* mutation is higher in hereditary breast cancer than in sporadic breast cancer [58].

### 2.5. EGFR and TNBC

Breast cancer can be divided into several genetically distinctive subtypes. One subtype, TNBC, characterized as negative for estrogen receptor (ER), progesterone receptor, and HER2, is associated with metastatic aggressiveness, a high rate of relapse, and shorter overall survival duration [59]. TNBC tumors are generally poorly differentiated, large, and heterogeneous; the majority (71–91%) are basal like, with overexpression of ‘basal’ keratins. TNBCs can be classified into seven molecular subtypes: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory, mesenchymal (M), mesenchymal stem-like, luminal androgen receptor (LAR), and unstable. A recent study showed that TNBC subtype and pathological complete response (pCR) status are significantly associated [60]. The BL1 subtype had the highest pCR rate (52%), whereas the BL2 and LAR subtypes had the lowest pCR rates (0% and 10%, respectively).

EGFR is overexpressed more often in TNBC than in breast cancer series that include multiple subtypes (60% vs. 25%, respectively) [61]. EGFR immunoreactivity has also shown significant correlation with worse prognosis in patients with TNBC [62]. These findings suggest that EGFR inhibitor may have an important role in targeted therapy for patients with this subtype.

Gene mutation rates in TNBC have been shown to be significantly higher compared with those in sporadic breast cancer not selected for subtype [63]. Mutated *TP53* causes inhibition of p63 protein, which leads to changes in the expression of genes such as *Dicer*, *DEPDC1*, *SHARP1*, and *cyclin G2* that are implicated in invasion and metastasis. Mutated *TP53* also causes the Rab-coupling protein-mediated recycling of integrins, EGFR, and MET proteins to promote the growth of cancer cells [64]. Therefore, targeting integrin pathways, EGFR, and MET pathways can be further pursued in TNBC. These data suggest that TNBC is the target population with most potential for detecting the *EGFR* mutation; simultaneously, *EGFR* mutation status might serve as a selection tool for patients likely to respond to EGFR-targeted treatment.

### 2.6. EGFR and IBC

IBC accounts for 3–5% of all breast cancers and is the most aggressive form of breast cancer. Compared with non-IBC locally advanced breast cancer, IBC has a worse prognosis and a distinctive pattern of early recurrence. Current treatment modalities for IBC are inadequate, and a better understanding of the biological features of the disease is needed to develop more effective interventions. IBC tumors are characterized by a high histologic grade and a high proliferation rate (e.g. elevated MIB1 expression, high S-phase proportion, high thymidine-labeling index); a high rate of ER-negative status; and overexpression of E-cadherin, p53, MUC1, RhoC, TIG1, and growth factor receptors such as HER2 and EGFR [65]. IBC is often associated with a 'basal-like' phenotype; high intratumoral microvessel density; high levels of tumor angiogenesis- and lymphangiogenesis-related factors such as vascular endothelial growth factor (VEGF)-A, -C, and -D, Flt-1, KDR, Tie1, and Tie2; and high expression of chemokines such as CCL3/MIP1A and CCL5/RANTES. An immunohistochemistry (IHC) analysis of IBC demonstrated HER2 overexpression in 21 of 44 patients (48%) and EGFR overexpression in 12 of 40 patients (30%). Significantly, EGFR overexpression was the predictive factor of worse outcome: the 5-year overall survival rate was significantly lower in women with EGFR-positive disease than in women with EGFR-negative disease ($p = .01$) [65]. Moreover, tumors that expressed EGFR, alone or in combination with CXCR4, were associated with a relatively higher incidence of bone recurrence [65]. The association between EGFR overexpression and increased risk of recurrence and death indicates that EGFR may represent a potential therapeutic target in IBC [66]. The inflammatory pathways in relationship with EGFR have been investigated in preclinical studies, and further studies are needed in clinical settings.

## 3. EGFR-targeted agents

We currently have two types of EGFR-targeted agents: mAbs (Table 1) and TKIs (Table 2), which have been evaluated in several studies. The primary action of mAbs is to bind to the extracellular domain of EGFR, which induces competitive antagonism, thus inhibiting receptor dimerization, autophosphorylation, and downstream signaling [15]. MAbs are classified into four types by class of action: murine, chimeric, humanized, and human [67]. In clinical research, humanized mAbs have provided new prospects for the treatment of breast cancer. In contrast, TKIs bind to intracellular domains of EGFR. TKIs are oral non-peptide quinazoline compounds homologous to adenosine triphosphate (ATP). This similarity allows them to compete for the ATP-binding domain of protein kinases, preventing phosphorylation and subsequent activation of signal transduction pathways, leading to apoptosis and decreasing cellular proliferation [68]. Over the past few years, EGFR-targeted therapies in breast cancer have had generally disappointing results. Here, we review the data for two mAbs (cetuximab and panitumumab; Table 3) and three TKIs (gefitinib, neratinib, and vandetanib; Table 4), focusing on clinical evidence and their potential mechanisms in breast cancer.

### 3.1. mAbs for breast cancer

**3.1.1. Cetuximab—**Cetuximab is the anti-EGFR mAb that has been most extensively studied in the clinic. It is a chimeric mAb specific for EGFR: it directly interacts with EGFR

and blocks the downstream pathway, which interferes with the proliferation of cancer cells. Early clinical data indicate promising activity with cetuximab in various combination regimens in patients with TNBC [69,70]. Blockade of EGFR promoted chemosensitization in breast cancer cell lines, providing an additional rationale for the combination of cetuximab with cytotoxic agents in breast cancer [71]. Cetuximab in combination with cisplatin as first-line treatment in patients with metastatic TNBC achieved a higher overall response rate (ORR) and longer progression-free survival (PFS) compared to cisplatin alone (ORR: 20% vs. 10%, respectively; median PFS: 3.7 vs. 1.5 months, respectively; odds ratio: 2.13; 95% confidence interval [CI]: 0.81–5.59; $p = .11$) [70]. Recent prospective studies tested combination therapy with cetuximab and ixabepilone for first-line treatment for advanced/metastatic TNBC. Similar ORR results were observed for ixabepilone monotherapy and ixabepilone/cetuximab combination treatment, 30% (95% CI: 16.6–46.5%) vs. 35.9% (95% CI: 21.2–52.8%), respectively. The median PFS was 4.1 months in both treatment groups [72]. These findings are superior to the previous subset analysis of cetuximab in combination with carboplatin or cisplatin in advanced TNBC. In an *in vitro* study, sequential application of anti-EGFR antibody and cytotoxic agent showed synergistic apoptotic effect [66]; however, in clinical studies, adding anti-EGFR antibody to cytotoxic therapy has not enhanced the effectiveness of treatment thus far. A Phase I study of cetuximab plus paclitaxel and then cetuximab plus docetaxel showed disappointing efficacy and unacceptable skin toxicity for patients with breast cancer [73]. Further research is warranted to establish the utility of combining an anti-EGFR antibody and cytotoxic agent.

**3.1.2. Panitumumab—**Panitumumab is a fully humanized IgG2 anti-EGFR mAb. It has a high affinity ($Kd = 5 \times 10^{-11}$ M) to the receptor. Panitumumab blocks EGF ligands and TGF-α binding to EGFR and thereby inhibits tumor growth and elicits both tumor regression and eradication of established tumors in murine xenograft tumor models [74].

To date, panitumumab has been evaluated in combination with chemotherapy in subjects with colorectal cancer, NSCLC, squamous cell carcinoma of the head and neck, and breast cancer. Two clinical studies were reported in breast cancer [75,76]. A Phase II trial in patients with metastatic TNBC had an ORR of 46% in 13 evaluable patients given weekly paclitaxel and carboplatin in combination with panitumumab [75]. The median time to best response was 2.4 months, and the median time to disease progression was 3.6 months. These results are consistent with other studies of response to cytotoxic chemotherapy in metastatic TNBC. On the other hand, a neoadjuvant trial of panitumumab in combination with FEC100 followed by docetaxel achieved a 46.8% pCR rate in patients with operable TNBC [76]. These results suggest the activity of panitumumab in TNBC, but we still lack predictive biomarkers that could help define large subsets of patients with a high probability of pCR. Currently, two Phase II trials that include panitumumab are ongoing in TNBC and IBC, which will allow further assessment of panitumumab combination treatments in biologically defined subgroups (ClinicalTrials. gov ID: NCT01036087 and NCT02593175).

Both cetuximab and panitumumab may exhibit more potent activity than that of small-molecule inhibitors in the *in vivo* setting, which would likely be attributable to antibody-dependent cell-mediated cytotoxicity induced by the mAbs' triggering of the Fc-RIII receptor on natural killer cells [77].

### 3.2. TKIs for breast cancer

**3.2.1. Gefitinib—**Gefitinib is a reversible EGFR TKI that was approved by the US Food and Drug Administration in 2003 for the treatment of advanced NSCLC with *EGFR* mutations. In preclinical breast cancer models, gefitinib inhibits growth of tumor cells with different expression levels of EGFR and HER2 [78–80]. However, the results of Phase I and II clinical trials were disappointing. A Phase II trial of gefitinib, 500 mg/day, in taxane- and anthracycline-pretreated metastatic breast cancer patients showed no significant clinical benefit, and 89.7% developed progressive disease [4]. In another study, gefitinib monotherapy did not show a favorable effect for advanced breast cancer. One suggested reason for failure was the lack of tumor dependence on the EGFR pathway [81].

On the other hand, some studies in ER-positive breast cancer patients reported favorable outcomes. In a Phase II randomized trial, 56 postmenopausal patients with ER-positive and EGFR-positive primary breast cancer were randomly assigned to receive gefitinib in combination with either anastrozole or placebo [82]. The gefitinib and anastrozole group had greater reduction of tumor. Ciardiello et al. reported that combination treatment with gefitinib and docetaxel for first-line therapy of metastatic breast cancer resulted in a response rate (complete plus partial responses) of 54%. Interestingly, the response was higher in patients with ER-positive tumors than in the rest of the breast cancer patients (70% vs. 21%, respectively) [83]. In another Phase II trial of 290 metastatic breast cancer patients with hormone receptor-positive disease, first-line treatment of gefitinib and docetaxel achieved good response [84]. In summary, the therapeutic efficacy of gefitinib and the population of breast cancer patients who may benefit from gefitinib treatment need further investigation and validation.

**3.2.2. Neratinib—**Neratinib, an oral irreversible inhibitor of pan-EGFR RTKs, interacts with the catalytic domain of EGFR, HER2, and HER4 [85]. Neratinib forms a covalent bond with cysteine residues in the ATP-binding pocket of these enzymes [86]. In preclinical studies, HER2-positive cell lines were more sensitive to neratinib than other triple-negative or luminal cell lines [87,88]. Neratinib-treated BT474 cells showed suppression of the MAPK and AKT pathways, inducing downregulation of cyclin D1 level, thereby inducing p27. The combination of neratinib and trastuzumab was more potent than trastuzumab alone or neratinib alone in reducing tumor size in both trastuzumab-sensitive and trastuzumab-resistant cell lines (SKBR3 and BT474, respectively) [88]. In addition, the reaction to neratinib was associated with HER2 and phosphorylated HER2 levels, but not with EGFR levels, *in vitro* [88]. Neratinib binds to cysteine residues Cys-773 and Cys-805 in EGFR and HER2, respectively [89]. Because of this particular authoritative work, higher specificity of the compound is accomplished.

Several clinical trials have shown promising activity of neratinib, particularly in HER2-positive breast cancer patients. In a Phase I clinical trial of neratinib, the dose-limiting toxicities included diarrhea, nausea/vomiting, and rash. The ORR observed in breast cancer patients was 32% (8 of 25 patients) and 39% (7 of 18 patients) with highly HER2-positive tumors (IHC of 3+) responded [90].

A Phase II trial of single-agent neratinib showed a 24% response rate in patients with metastatic breast cancer who had progression following trastuzumab treatment and a 56% response rate when it was given as first-line therapy [91]. In addition, Martin et al. reported that the PFS duration did not significantly differ between two treatment groups, neratinib vs. the combination of lapatinib plus capecitabine, for HER2-positive locally advanced or metastatic breast cancer [92].

Of interest is to determine whether neratinib could effect a preferable response in patients with *EGFR* mutation. A clinical study of neratinib's efficacy is under way in patients with solid tumors with *EGFR* mutation or amplification, including breast cancer (ClinicalTrials.gov ID: NCT01953926). A recent single-arm Phase II trial of neratinib for HER2-mutated, non-amplified metastatic breast cancer showed a 36% (90% CI: 15–61) clinical benefit rate (complete response, partial response, or stable disease ≥6 months) in a heavily treated patient population [93]. This trial suggested that the cross talk between the hormone receptor and HER2 signaling pathways can lead to endocrine resistance. To test how *HER2*-mutant cancer responds to treatment with neratinib, a Phase II study of neratinib alone and in combination with fulvestrant in metastatic HER2 non-amplified but *HER2*-mutant breast cancer is currently ongoing (ClinicalTrials.gov ID: NCT01670877).

### 3.3. Drugs that target multiple molecular pathways

Recently, several preclinical and clinical trials have studied EGFR inhibitors that target multiple molecular pathways at the same time. For instance, vandetanib (ZD6474) is a multi-kinase inhibitor that suppresses VEGF receptor 2 (VEGFR-2), RET, and EGFR [94]. It is approved for the treatment of symptomatic or progressive medullary thyroid cancer. VEGFs induce tumor-associated angiogenesis through activation of its cognate receptors of TKIs, VEGFR-1, and VEGF-2. VEGFs activate several intracellular signaling cascades, including the RAS/RAF/ERK and PI3K/AKT pathways [95]. Vandetanib monotherapy is well tolerated, but its therapeutic efficacy has been limited in patients with previously treated metastatic breast cancer; there were no objective responses in a Phase II trial [96]. The efficacy of vandetanib in combination with chemotherapeutic agents has not been promising. A randomized Phase II study of 64 patients assessed the efficacy and safety of vandetanib in combination with docetaxel vs. docetaxel alone as second-line treatment for advanced breast cancer [97]. The combination of vandetanib and docetaxel was generally well tolerated; however, no clinical benefit was observed (hazard ratio 1.19; two-sided $p = .59$). A Phase II trial was conducted to evaluate the benefit of fulvestrant plus vandetanib vs. fulvestrant plus placebo in postmenopausal women with bone-only or bone-predominant, hormone receptor-positive metastatic breast cancer [98]. There was no difference between the two groups for PFS (hazard ratio: 0.95; 95% CI: 0.65–1.38) or overall survival (hazard ratio: 0.69; 95% CI: 0.37–1.31). The level of urine N-telopeptide, a biomarker of bone turnover, significantly decreased with the fulvestrant plus vandetanib treatment. Based on these trials, it is difficult to determine whether vandetanib has clinical benefit for breast cancer despite its antitumor activity in preclinical studies. However, this lack of antitumor activity in clinical trials could be explained by an adaptive mechanism of the tumor. Specifically, the activation of signaling pathways occurs in the early stage of tumor progression and differentiation, and it may be fundamentally adjusted by treatment with targeted agents and endocrine treatments [99].

## 4. Conclusion

It is important to identify the population of breast cancer patients in whom a preferable outcome for EGFR-targeted drugs can be achieved. Previous clinical studies have suggested that TNBC, basal-like, and inflammatory breast cancer might serve as good candidates for EGFR-targeted therapy. In addition, studies are under way to identify *EGFR* mutations or molecular alterations that could affect the response to EGFR-targeted therapy. An increased molecular and genomic approach will be needed to select patients who respond to EGFR-targeted therapy for breast cancer.

## 5. Expert opinion

Despite promising effects of EGFR-targeted therapy for patients with TNBC, basal-like breast cancer, and IBC, predictive biomarkers have not been identified to select appropriate breast cancer patients who may benefit from this therapy. Efforts should be made to establish a hypothesis-oriented clinical trial to identify molecular prognosis and predictive biomarkers. Analysis of gene expression by RNA sequencing and of cytokine expression in blood samples will allow us to identify novel predictors of pCR, which can be tested and validated while a clinical trial is ongoing. Analysis of changes in tumor gene mutation status during EGFR-targeted therapy could provide insights into tumor biology and drug resistance. Currently, adaptive biopsy-based studies to analyze metabolites and changes in expression of significant pathway molecules are being evaluated. Mechanistic studies to investigate the dynamic interaction between the EGFR pathway and non-canonical pathways (e.g. the inflammatory pathway) are warranted. Targeting several pathways simultaneously is critical; we need to develop novel combinations of EGFR-targeted therapy with other agents, possibly targeting the metastatic process.

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## Article highlights

- Epidermal growth factor receptor (EGFR) targeted treatment has been evaluated but has not shown a clear clinical benefit for breast cancer.
- Recent studies have indicated that EGFR targeted therapy for breast cancer has some promising effects for patients with specific types of breast cancer, such as triple-negative, basal-like, and inflammatory breast cancer.
- Changes in tumor genomic expression status during EGFR targeted therapy can provide insights into tumor biology and drug resistance.
- The analysis of gene expression by RNA sequencing and of cytokine expression in blood samples will allow us to identify novel predictors for response.
- Mechanistic studies to investigate the dynamic interaction between the EGFR pathway and non-canonical pathways (e.g. the inflammatory pathway) are warranted.

This box summarizes key points contained in the article.

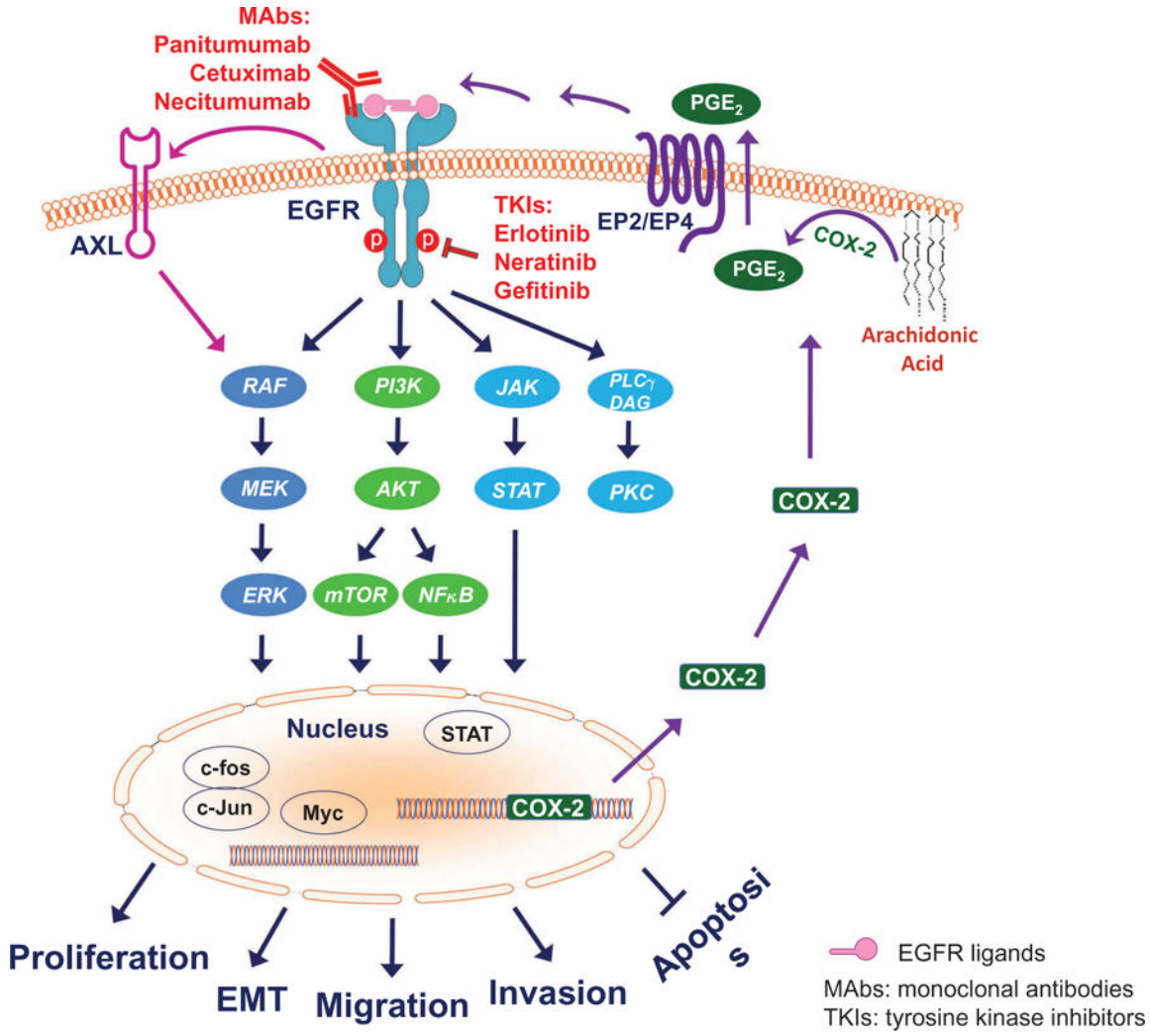


**Figure 1.**
The downstream pathways which mediate the signals of EGFR receptor activation.

**Table 1**

Monoclonal antibodies (mAbs) against epidermal growth factor receptor under investigation for treatment of breast cancer.

| MAb | Class of action | Phase of study |
|---|---|---|
| Cetuximab | Chimeric MAb | Phase I, II |
| Panitumumab | Humanized MAb | Phase II |
| GA201 | MAb | Phase I, solid tumors |
| Nimotuzumab | Humanized MAb | Phase I |
| Matuzumab | Humanized MAb | Preclinical |
| Necitumumab | Humanized MAb | Phase II |

## Table 2

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors under investigation for treatment of breast cancer.

| TKI | Target | Class of action | Phase of study |
|---|---|---|---|
| Gefitinib | EGFR | Reversible TKI | Phase I, II |
| Erlotinib | EGFR | Reversible TKI | Phase I, II |
| Aderbasib | EGFR | Reversible TKI | Phase II |
| AE37 | EGFR | Reversible TKI | Phase II |
| AZD4769 | EGFR | TKI | Phase I, solid tumors |
| Lapuleucel-T (APC8024) | EGFR | Designed to stimulate cellular immune responses against HER2/neu | Phase I |
| CL-3877785 | EGFR | Irreversible TKI | Preclinical |
| Lapatinib | EGFR, ErbB2 | Reversible TKI | In clinical use |
| Afatinib (BIBW 2992) | EGFR, ErbB2 | Irreversible TKI | Phase II |
| S222611 | EGFR, ErbB2 | Reversible TKI | Phase I, solid tumors |
| TAK-285 | EGFR, ErbB2 | TKI | Phase I, solid tumors |
| AV412 | EGFR, ErbB2 | Irreversible TKI | Phase I |
| PKI-166 | EGFR, ErbB2 | TKI | Phase I, solid tumors |
| Varlitinib (ARRY-334543) | EGFR, ErbB2, ErbB4 | Reversible TKI | Phase II |
| BMS-599626 | EGFR, ErbB2, ErbB4 | Reversible TKI | Phase I |
| EKB-569 | EGFR, ErbB2, ErbB4 | Irreversible TKI | Phase I |
| Dacomitinib (PF-299804) | EGFR, ErbB2, ErbB4 | Irreversible TKI | Phase I, solid tumors |
| Sapitinib (AZD8931) | EGFR, ErbB2, ErbB3 | Reversible TKI | Phase I, solid tumors |
| Vandetanib (ZD6474) | EGFR, VEGF, RET | TKI | Phase I, II |
| CUDC-101 | EGFR, ErbB2, HDAC | Irreversible TKI | Phase I, solid tumors Phase Ib |
| Neratinib (HKI-272) | EGFR, ErbB2, ErbB4 | Irreversible TKI | Phase I, II, III |
| Canertinib (CI-1033) | EGFR, ErbB2, ErbB4 | Irreversible TKI | TKI Phase I, II |
| BMS-690514 | EGFR, ErbB2, ErbB4, VEGFR1-3 | Irreversible TKI | Phase I, solid tumors |
| Tesevatinib (XL647) | EGFR, ErbB2, EphB4, VEGF | Reversible TKI | Phase I |
| AEE788 | EGFR, ErbB2, VEGFR | Reversible TKI | Phase I |
| ARRY-380 | ErbB2, AKT | Reversible TKI | Phase I |

HDAC: histone deacetylase; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; TKI: tyrosine kinase inhibitor. Permission to use from the Springer, license number 4017520234602.

**Table 3**

Clinical trials of monoclonal antibodies (MAbs) cetuximab and panitumumab for breast cancer.

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
|---|---|---|---|---|---|---|---|---|---|---|
| Cetuximab | Combination | Chimeric MAb | 2015 | Tredan | 79 (Arm 1: 40; Arm 2: 39) | Phase II, randomized trial | First-line treatment for advanced/metastatic TNBC | Arm 1: ixabepilone (40 mg/m$^2$ every 21 days). Arm 2: ixabepilone (40 mg/m$^2$ every 21 days) with cetuximab (400 mg/m$^2$ loading dose, followed by 250 mg/m$^2$ once weekly). | RR of ixabepilone monotherapy and ixabepilone with cetuximab combination therapy | Arm 1 vs. Arm 2: RR 30% (95% CI: 16.6–46.5) vs. 35.9% (95% CI: 21.2–52.8) |
| Cetuximab | Combination | Chimeric MAb | 2014 | Nechushtan | 18 | Phase I/II | Metastatic TNBC | Cetuximab: initial 400 mg/m$^2$ loading dose (200 mL/m$^2$) administered over a period of 120 min (maximum infusion rate of 5 mL/min), followed by a weekly dose of 250 mg/m$^2$ (125 mL/m$^2$) administered over a period of 60 min (maximum infusion rate of 5 mL/min). Paclitaxel: 80 mg/m$^2$ administered weekly. | Objective tumor response, tumor marker, clinical response, TTF | Median TTF: 6 months. OS: 12 months. CR: 5.6% (1 of 18). PR: 22.2% (4 of 18). SD: 27.8% (5 of 18). |
| Cetuximab | Combination | Chimeric MAb | 2013 | Baselga | 115 | Phase II | Metastatic TNBC | Patients who had received no more than one previous chemotherapy regimen were randomly assigned on a 2:1 schedule to | Primary: ORR; secondary: PFS, OS | ORR: 20% (95% CI: 13–29) for cisplatin + cetuximab and 10% (95% CI: 4–21) for cisplatin alone |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | receive no more than six cycles of cisplatin (75 mg/m$^2$ on day 1, every 3 weeks, for six cycles) + cetuximab (400 mg/m$^2$ followed by 250 mg/m$^2$ once weekly) or cisplatin alone. Patients receiving cisplatin alone could switch to cisplatin + cetuximab or cetuximab alone on disease progression. | | (OR: 2.13; 95% CI: 0.81–5.59; $p$ = .11). Cisplatin + cetuximab resulted in longer PFS compared with cisplatin alone (median: 3.7 vs. 1.5 months; HR: 0.67; 95% CI: 0.47–0.97; $p$ = .032). Corresponding median OS was 12.9 vs. 9.4 months (HR: 0.82; 95% CI: 0.56–1.20; $p$ = .31). |
| Cetuximab | Combination | Chimeric MAb | 2012 | Carey | 102 | Phase II | Metastatic TNBC | Cetuximab (400 mg/m$^2$ loading dose, then 250 mg/m$^2$ per week IV), with carboplatin (AUC of 2, once per week IV) added after progression or as concomitant therapy from the beginning. | RR | RRs were 6% (2 of 31) for cetuximab alone; 16% (4 of 25) for cetuximab + carboplatin added after progression; and 17% (12 of 71) for those treated from the beginning with cetuximab + carboplatin; of this latter group, 31% of patients responded or had prolonged disease stabilization. |
| Cetuximab | Mono | Chimeric MAb | 2011 | Ho | 27 | Phase I | Solid tumors (breast cancer, $n$ = 2) | Cetuximab (400 mg/m$^2$ IV loading dose and 250, | DLT, MTD | Cetuximab administered at 400 mg/m$^2$ |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | 300, 350, or 400 mg/m$^2$ weekly IV maintenance) | | IV as a loading dose with weekly maintenance dose of 400 mg/m$^2$ is feasible and well tolerated. |
| Cetuximab | Combination | Chimeric MAb | 2006 | Modi | 12 | Phase I | Advanced breast cancer | Group 1: cetuximab 50 mg/m$^2$ and paclitaxel 175 mg/m$^2$, every 3 weeks; Group 2: cetuximab 100 mg/m$^2$ and paclitaxel 175 mg/m$^2$, every 3 weeks; Group 3: cetuximab 100 mg/m$^2$ and paclitaxel 80 mg/m$^2$, weekly; Group 4: cetuximab 200 mg/m$^2$ and paclitaxel 80 mg/m$^2$, weekly. | Feasibility of combination treatment (cetuximab +paclitaxel) | Ten of 12 patients were evaluable for response, and of these, 2 patients experienced SD, and 8 patients experienced disease progression. Two of 6 patients on the second cohort (cetuximab 100 mg/m$^2$) developed DLTs, presenting as Grade 3 rash. |
| Cetuximab | Combination | Chimeric MAb | 2000 | Baselga | 52 | Phase I | Advanced tumors (overexpressing EGFR) | Cetuximab as a single dose ($n$ = 13), weekly multiple doses ($n$ = 17), and weekly multiple doses with cisplatin ($n$ = 22). Cetuximab dose levels were 5, 20, 50, and 100 mg/m$^2$. | PK, toxicity | For cetuximab + cisplatin, 69% (9 of 13) patients treated with antibody doses ≥50 mg/m$^2$ completed 12 weeks of therapy, and 2 PRs were observed. |
| Panitumumab | Combination | Humanized MAb | 2015 | Cowherd | 14 | Phase II | Metastatic or locally advanced TNBC | Paclitaxel (80 mg/m$^2$) and carboplatin (AUC of 2) on days 1, 8, and 15 and panitumumab (6 mg/kg) on days 1 | RR, safety | ORR (CR + PR): 46% |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | and 15 for a cycle length of 28 days | | |
| Panitumumab | Combination | Humanized MAb | 2014 | Nabholtz | 62 | Phase II | Operable TNBC | Treatment in this multicentric neoadjuvant pilot study consisted of panitumumab (9 mg/kg) for eight cycles every 3 weeks combined with four cycles of 5-fluorouracil, epidoxorubicin, and cyclophosphamide (FEC100: 500/100/500 mg/m$^2$) every 3 weeks, followed by four cycles of docetaxel (100 mg/m$^2$) every 3 weeks. | Rate of pCR, according to Chevallier's classification | pCR: 46.8% (95% CI: 32.5–61.1). The association of high EGFR and low cytokeratin 8/18 expression in tumor cells and high density of CD8+ tumor-infiltrating lymphocytes on the other hand were significantly predictive of pCR. |

CI: confidence interval; TTF: time-to-treatment failure; RR: response rate; ORR: overall response rate; TNBC: triple-negative breast cancer; PFS: progression-free survival; OS: overall survival; IV: intravenous; DLT: dose-limiting toxicity; MTD: maximum tolerated dose; OR: odds ratio; CR: complete response; PR: partial response; SD: stable disease; pCR: pathological complete response; AUC: area under the curve; PK: pharmacokinetics; HR: hazard ratio; EGFR: epidermal growth factor receptor.

**Table 4**

Clinical trials of epidermal growth factor receptor (EGFR) family tyrosine kinase inhibitors (gefitinib, neratinib, and vandetanib) for breast cancer.

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
|---|---|---|---|---|---|---|---|---|---|---|
| Gefitinib | Mono | EGFR | 2014 | Kalykaki | 24 | Phase II | Metastatic breast cancer | Gefitinib (250 mg/day) given for a minimum of 3 months | Efficacy by quantitative analysis of CK-19 mRNA-circulating tumor cells | After the first and the second treatment cycles; a median reduction of 96.4% and 94.1% in CTC count was observed in 11 (64.7%) and 12 (70.6%) of patients, and total CTC numbers declined by 73% and 44%. |
| Gefitinib | Combination | EGFR | 2016 | Tryfonidis | 71 | Phase II | Advanced breast cancer | Experimental: anastrozole (1 mg/day) + gefitinib (A/G) (250 mg). Active comparator: anastrozole + placebo (A/P). In both cases, treatment administered until documented disease progression or unacceptable toxicity as judged by the responsible physician or until patient refusal. | PFS at 1 year | PFS rate at 1 year: A/G arm (36 patients), 32%; A/P arm (35 patients), 32%. |
| Gefitinib | Combination | EGFR | 2012 | Carlson | 148 | Phase II | MBC | Experimental Arm I: oral anastrozole and oral gefitinib (initial dose was 500 mg orally daily, due to high rate of diarrhea, starting dose was reduced to 250 mg orally daily) once daily on days 1–28. Experimental Arm II: fulvestrant intramuscularly on day 1 and oral gefitinib once daily on days 1–28. For both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. | CBR | 141 eligible subjects were enrolled. Anastrozole + gefitinib ($n = 72$) had a CBR of 44% (95% CI: 33–57); fulvestrant + gefitinib ($n = 69$), 41% (95% CI: 29–53). |
| Gefitinib | Combination | EGFR | 2007 | Denninson | 33 | Phase II | Advanced breast cancer | Docetaxel (75 mg/m$^2$ IV every 3 weeks) combined with gefitinib (250 mg orally daily) until disease progression or withdrawal criteria are met. | CBR, safety profile of the combination of gefitinib and docetaxel as measured by the frequency and severity of adverse events. | CBR: 51.5% (95% CI: 33.5–69.2). Median duration of clinical benefit: 10.9 months (95% CI: 6.0–17.6 months). Neutropenia in 43% of patients. |
| Gefitinib | Combination | EGFR | 2012 | Somlo | 31 (Phase I, 9; Phase II, 22) | Phase I/II | Stage IV HER2-positive MBC | Gefitinib (250 mg daily or 250 mg daily on days 2 through 14, depending on study findings); trastuzumab (cycle 1 loading dose of 8 mg/kg, followed by 6 mg/kg every 3 weeks for subsequent cycles); and docetaxel (75 mg/m$^2$ every 3 weeks or 60 mg/m$^2$ every 3 weeks, depending on study findings). | Phase I: MTD. Phase II: primary end point, PFS. | MTD: gefitinib, 250 mg on days 2–14; trastuzumab, 6 mg/kg; and docetaxel, 60 mg/m$^2$ every 21 days. Median PFS: 12.7 months. CR: 18%. PR: 46%. SD: 29%. |
| Gefitinib | Combination | EGFR | 2011 | Bernsdorf | 181 | Phase II | Estrogen receptor-negative operable breast cancer | Specimens from patients randomized and completing treatment in the NICE | pCR | Pathological CR was observed in 17% (12 of 71) of patients |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | trial (four cycles of neoadjuvant epirubicin and cyclophosphamide + 12 weeks of either gefitinib [250 mg daily] or placebo). Cases with available ER status both at baseline and after neoadjuvant treatment were eligible for this study. | | treated with gefitinib and 12% (9 of 73) of patients treated with placebo (4.57% difference; 95% CI: −7.19 to 6.33; $p = .44$). |
| Gefitinib | Combination | EGFR | 2011 | Massarweh | 15 | Phase II | Newly diagnosed estrogen receptor-positive breast cancer | Experimental: subjects randomized to gefitinib on day 1 in combination with Arimidex and Faslodex.<br>Active comparator: subjects randomized to Iressa on day 21 in combination with Arimidex and Faslodex. | Clinical RR | In the 12 evaluable patients; clinical CRs ($n = 2$, 17%), PRs ($n = 3$, 25%), SD ($n = 5$, 41%), PD ($n = 2$, 17%). |
| Gefitinib | Combination | EGFR | 2011 | Osborne | 290 (stratum 1, $n = 206$; stratum 2, $n = 84$) | Phase II | Hormone receptor-positive MBC | Patients were randomized (1:1) to receive tamoxifen (20 mg/day orally) plus gefitinib (250 mg/day orally) or tamoxifen (20 mg/day orally) plus placebo.<br>Stratum 1: Patients with newly metastatic disease or recurrence after adjuvant tamoxifen.<br>Stratum 2: Patients with recurrence during/after adjuvant aromatase inhibitor or after failed first-line aromatase inhibitor. | Stratum 1: PFS.<br>Stratum 2: CBR. | Stratum 1 ($n = 206$): PFS HR (gefitinib vs. placebo): 0.84 (95% CI: 0.59–1.18). Median PFS: 10.9 months for gefitinib vs. 8.8 months for placebo. CBR: 50.5% for gefitinib and 45.5% for placebo. Stratum 2 ($n = 84$): CBR: 29.2% for gefitinib and 31.4% for placebo. |
| Gefitinib | Mono | EGFR | 2010 | Gutteridge | 54 | Phase II | ER-positive patients who had acquired tamoxifen resistance and patients with ER-negative tumors | Oral gefitinib (500 mg/day) | Clinical benefit rate (objective response or stable disease >24 weeks) | Gefitinib was well tolerated. The CBR (objective response or SD >24 weeks) rates were 33.3% overall (18 of 54); 53.6% in ER-positive/ tamoxifen-resistant patients ($n = 28$); and 11.5% in ER-negative patients ($n = 26$). |
| Gefitinib | Combination | EGFR | 2010 | Cristofanilli | 174 | Phase II | Hormone receptor-positive MBC | 43 patients were randomized to anastrozole (1 mg/day orally, standard approved dose) + gefitinib (250 mg/day orally), and 50 patients were randomized to anastrozole + placebo | PFS | PFS for patients receiving anastrozole + gefitinib was longer than for patients receiving anastrozole + placebo (HR [gefitinib vs. placebo]: 0.55; 95% CI: 0.32–0.94; median PFS: 14.7 vs. 8.4 months). |
| Gefitinib | Mono | EGFR | 2009 | Green | 66 | Phase II | Advanced breast cancer | Group 1: patients with hormone receptor-positive, hormone-resistant advanced breast cancer for whom chemotherapy was not currently indicated. | CBR | Group 1: CBR 0%. Group 2: CBR 7.7% (95% CI: 0.9–25.1). |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | Group 2: patients with hormone receptor-negative advanced breast cancer.<br>Both groups received gefitinib monotherapy (500 mg/day). | | |
| Gefitinib | Combination | EGFR | 2009 | Mayer | 19 | Phase I | MBC | Sequential cohorts ($n$ = 3) received gefitinib and escalating capecitabine on a 14-day-on/7-day-off schedule, with a validation cohort ($n$ = 10) at the MTD. | MTD, safety | 19 patients were treated for a median of five cycles. No patients in sequential cohorts experienced DLT; capecitabine MTD was 2000 mg/m$^2$/day when paired with daily gemcitabine (250 mg). |
| Gefitinib | Mono | EGFR | 2010 | Campos | 35 | Phase I | Refractory gynecological malignancies or MBC | Escalating doses of liposomal doxorubicin (Level 1, 25 mg/m$^2$, 30 mg/m$^2$; Level 2, 35 mg/m$^2$; Level 3, 40 mg/m$^2$ IV) were administered every 4 weeks with gefitinib (250 mg/day orally). | Safety and efficacy of the combination of liposomal doxorubicin and gefitinib | Dose level 3 was determined to be the MTD. |
| Gefitinib | Combination | EGFR | 2005 | Polychronis | 56 | Phase II | Postmenopausal patients with ER-positive and EGFR-positive primary breast cancer | 27 women were randomly assigned to gefitinib (250 mg/day orally) + anastrozole (1 mg/day orally), and 29 women to gefitinib (250 mg/day orally) + placebo. | Primary outcome was inhibition of tumor-cell proliferation, as measured by Ki-67 antigen labeling index. | Patients assigned gefitinib + anastrozole had a greater reduction from pretreatment values in proliferation-related Ki-67 than did those assigned gefitinib + placebo (mean % reduction: 98.0 [95% CI: 96.1–98.9] vs. 92.4 [95% CI: 85.1–96.1]; difference between groups: 5.6% [95% CI: 5.1–6.0], $p$ = .0054). |
| Gefitinib | Combination | EGFR | 2008 | Arteaga | 35 | Phase I/II | Metastatic HER2-positive breast cancer | Phase I: trastuzumab (2 mg/kg/week) + gefitinib (500 mg/day).<br>Phase II: trastuzumab (2 mg/kg/week) + gefitinib (250 mg/day). | Increase the proportion of PFS from 50% to 65% at 6 months in chemotherapy-naive patients and from 50% to 70% at 3 months in patients previously treated with chemotherapy in the metastatic setting.<br>Safety and efficacy of gefitinib combination with trastuzumab. | Phase I: MTD for gefitinib was 250 mg/day.<br>Phase II: CR: 1 patient. PR: 2 patients. SD: 6 patients. CBR: 28%. |
| Gefitinib | Mono | EGFR | 2005 | Baselga | 31 | Phase II | Advanced breast cancer | Gefitinib (500 mg/day) | ORR | Of the 31 patients, 12 (38.7%) had SD, of whom 10 (32.3%) were stable for ≥3 months, 6 (19.4%) were stable for ≥4 months, and 3 (9.7%) were stable for ≥6 months. No CR and PR. |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| Gefitinib | Combination | EGFR | 2007 | Smith | 206 | Phase II | Postmenopausal early (stage I-IIIB) hormone receptor-positive breast cancer | All patients received anastrozole (1 mg daily for 16 weeks). In addition, patients were randomly assigned at a ratio of 2:5:5 to receive gefitinib (250 mg/day orally for 16 weeks); placebo (1 tablet/day orally for 2 weeks for 2 weeks) followed by gefitinib (250 mg/day orally for 14 weeks); or placebo (1 tablet/day orally for 16 weeks). | Biologic change in proliferation as measured by Ki-67 at 2 and 16 weeks | Mean changes in Ki-67 with anastrozole + gefitinib versus anastrozole alone were −77.4% and −83.6%, respectively, between baseline and 16 weeks (geometric mean ratio: 1.37; 95% CI: 0.79–2.39; $p = .26$); −80.1% and −71.3% between baseline and 2 weeks (geometric mean ratio: 0.70; 95% CI: 0.39–1.25; $p = .22$); and −19.3% and −43% between 2 and 16 weeks (geometric mean ratio: 1.42; 95% CI: 0.86–2.35; $p = .16$). |
| Gefitinib | Combination | EGFR | 2008 | Guarneri | 90 | Phase II | Operable (stage II-IIIA) breast cancer | Randomized to receive epirubicin (90 mg/m$^2$) and paclitaxel (175 mg/m$^2$) on day 1 + gefitinib (250 mg daily) from days 5 to 16 (Arm A, intermittent); gefitinib (250 mg daily) from days 1 to 21 (Arm B, continuous); or placebo (Arm C). | *In vivo* effect of adding gefitinib to preoperative chemotherapy on EGFR-dependent p42/44 MAPK and other biological variables. | pCR was observed in 4 patients. No significant differences in the expression of p42/44 MAPK, EGFR, p-EGFR, VEGFR2, proliferation index, and apoptosis were observed when comparing the combined Arms A + B vs. C and comparing Arm A vs. B. |
| Gefitinib | Combination | EGFR | 2005 | Gasparini | 15 | Phase I | MBC | Gefitinib (250 mg/day orally). The starting dose of epirubicin was 20 mg/m$^2$. Escalating dose levels of epirubicin were planned by increments of 5 mg/m$^2$ per level, up to the MTD. | MTD | The recommended dose of epirubicin for Phase II studies was 30 mg/m$^2$ in combination with gefitinib at the daily dose of 250 mg. PK did not identify any biomarker predictive of response. |
| Gefitinib | Combination | EGFR | 2005 | von Minckwitz | 58 | Phase II | Taxane- and anthracycline-pretreated MBC | Gefitinib (500 mg/day) | CBR and safety | PR: 1.7% (1 of 58); progressive disease: 89.7% (52 of 58). Median time to progression: 61 days (95% CI: 54–82 days). Median OS time: 357 days (95% CI: 257–441 days). There was no correlation between EGFR expression and response. |
| Gefitinib | Combination | EGFR | 2006 | Ciardiello | 41 | Phase II | MBC, first-line treatment | All patients received oral gefitinib (250 mg/day). IV docetaxel was given at 75 mg/m$^2$ (first 14 patients) or 100 mg/m$^2$ (the following 27 patients) on day 1 of each 3-week cycle. | Activity and safety of gefitinib in combination with docetaxel | CR or PR was observed in 22 of 41 patients with a 54% RR (95% CI: 45–75). |
| Gefitinib | Combination | EGFR | 2005 | Fountzilas | 68 | Phase I/II | Advanced breast cancer | Paclitaxel (175 mg/m$^2$ over 3 h), carboplatin (AUC of 6, three times a week) for six cycles, and gefitinib (250 mg/day orally) | Phase I: safety of the combination in this patient population at a set dose. Phase II: ORR. | Sixty-three (92.7%) patients were evaluable for response; 9 (13.2%) had CRs, 30 (44.1%) had PRs, 21 (30.9%) had SD, |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| | | | | | | | | | | and 3 (4.4%) had disease progression. |
| Gefitinib | Combination | EGFR | 2008 | Gioulbasanis | 35 | Phase I/II | MBC pre-treated with taxane and anthracycline chemotherapy | Oral gefitinib (250 mg/day) continuously, combined with IV gemcitabine (1000 mg/m$^2$) + vinorelbine (25 mg/m$^2$) on day 1, every 2 weeks. | Phase I: safety and tolerability.<br>Phase II: efficacy of the combination of gefitinib with gemcitabine + vinorelbine. | In an intent-to-treat analysis, the overall RR was 12% (95% CI: 0–24.7), the median time to tumor progression was 3.5 months (range: 1.0–11.5 months), and the median OS was 10.4 months (range 1.0–46.0 months). |
| Gefitinib | Mono | EGFR | 2005 | Goss | 28 | Phase I | Solid tumors | Gefitinib: 150–800 mg/day. PK sampling was performed on days 8, 15, 22, and 29; toxicity assessment every 28 days. | MTD, DLT, the biologically active dose, PK. | Gefitinib at a dose of 800 mg/day was tolerable although some patients required dose modification for diarrhea. Doses above 250 mg/day demonstrate biologic activity and could be considered for future study in a variety of EGFR-positive tumor types. |
| Neratinib | Combination | Pan-EGFR | 2013 | Chow | 115 | Phase I/II | Solid tumors and breast cancer | Part 1, dose level 1: neratinib (160 mg/day orally) + paclitaxel (80 mg/m$^2$ weekly IV).<br>Part 1, dose level 2: neratinib (240 mg/day orally) + paclitaxel (80 mg/m$^2$ weekly IV).<br>Part 2, expanded MTD cohort, Arm A: subjects with MBC who have not received more than one prior cytotoxic chemotherapy treatment regimen for metastatic disease will receive MTD of neratinib in combination with paclitaxel.<br>Part 2, expanded MTD cohort, Arm B: subjects with MBC who have not received more than three prior cytotoxic chemotherapy treatment regimens for metastatic disease will receive MTD of neratinib in combination with paclitaxel. | Part 1: MTD of neratinib + paclitaxel in patients with solid tumors.<br>Part 2: safety, efficacy, and PK of the combination at the MTD in patients with HER2-positive breast cancer. | Part 1: MTD: oral neratinib 240 mg once daily + IV paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of each 28-day cycle.<br>Part 2 ($n$ = 102): overall median treatment duration: 47.9 weeks (range: 0.1–147.3 weeks). ORR: 73% (95% CI: 62.9–81.2). CR: 7 patients (7%). Median PFS: 57.0 weeks (95% CI: 47.7–81.6 weeks). |
| Neratinib | Combination | Pan-EGFR | 2013 | Jankowitz | 21 | Phase I | HER2-positive MBC | Paclitaxel (80 mg/m$^2$ IV) on days 1, 8, and 15 every 28 days until disease progression.<br>Biological: trastuzumab (4 mg/kg IV loading dose, then 2 mg/kg IV) weekly until disease progression.<br>Drug: neratinib (dose level 1: 120 mg/day orally; dose level 2: 160 mg/day orally; dose level 3: 240 mg/day orally; dose level 4: 200 mg/day orally). | Safety and tolerability of the three-drug combination. | RP2D of neratinib with trastuzumab and paclitaxel: 200 mg/day.<br>Grade 3/4 events: diarrhea (38%), dehydration (14%), electrolyte imbalance (19%), and fatigue (19%). |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
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| Neratinib | Combination | Pan-EGFR | 2014 | Saura | 105 | Phase I/II | Solid tumors and HER2-positive metastatic or locally advanced breast cancer | Neratinib (240 mg, continuous daily) + capecitabine (1500 mg/m$^2$ on days 1–14 of each 21-day cycle) | Phase I: MTD of neratinib + capecitabine in patients with solid tumors. Phase II: safety and efficacy in patients with HER2-positive MBC. | Phase I: MTD ($n$ = 33): neratinib 240 mg/day + capecitabine 1500 mg/m$^2$/day. Phase II: ORR: 64% (39 of 61) in patients with no prior lapatinib exposure and 57% (4 of 7) in patients previously treated with lapatinib. Median PFS: Phase I, 40.3 weeks; Phase II, 35.9 weeks. |
| Neratinib | Combination | Pan-EGFR | 2013 | Awada | 92 | Phase I/II | Solid tumors and MBC | Neratinib (160 or 240 mg/day) + vinorelbine (25 mg/m$^2$ on day 1 and day 8 of a 21-day cycle). | Phase I: MTD of oral neratinib (160 or 240 mg/day) + vinorelbine in patients with solid tumors. Phase II: safety, ORR, and PK. | Phase I ($n$ = 12): MTD: neratinib 240 mg + vinorelbine 25 mg/m$^2$. Phase II ($n$ = 79): ORR: 41% (no prior lapatinib) and 8% (prior lapatinib). There was no evidence of PK interaction between neratinib and vinorelbine. |
| Neratinib | Mono | Pan-EGFR | 2013 | Martin | 233 | Phase II | HER2-positive advanced breast cancer | Experimental ($n$ = 117): neratinib (tablets, 240 mg once per day). Active comparator ($n$ = 116): lapatinib (tablets 1250 mg once per day) + capecitabine (tablets 2000 mg/m$^2$ given in two evenly divided daily doses for first 14 days of each 21-day cycle). | PFS | The non-inferiority of neratinib was not demonstrated when compared with lapatinib + capecitabine (HR: 1.19; 95% CI: 0.89–1.60; non-inferiority margin: 1.15). Median PFS: 4.5 months for neratinib vs. 6.8 months for lapatinib + capecitabine. Median OS: 19.7 months vs. 23.6 months, respectively. |
| Neratinib | Combination | Pan-EGFR | 2013 | Jankowitz | 21 | Phase I | Metastatic HER2-positive breast cancer | Neratinib (120–240 mg/day) with trastuzumab (4 mg/kg IV loading dose, then 2 mg/kg IV weekly) and paclitaxel (80 mg/m$^2$ IV on days 1, 8, and 15 of a 28-day cycle). | MTD, RP2D, efficacy, and tolerability | RP2D: 200 mg/day. CR and PR: 8 patients (38%), with a clinical benefit of CR + PR + SD ≥24 weeks in 11 patients (52%). Median time to disease progression: 3.7 months. |
| Neratinib | Combination | Pan-EGFR | 2014 | Gandhi | 63 | Phase I | Solid tumors | 4-by-4 dosing plan. The dose level for each cohort of two patients was determined by a nonparametric up-and-down design. Two initial cohorts of two patients each were simultaneously enrolled to receive daily doses of: (1) 160 mg neratinib + 15 mg temsirolimus; or (2) 120 mg neratinib + 25 mg temsirolimus. | Toxicity, RP2D | Two MTD combinations were identified: 200 mg neratinib/25 mg temsirolimus and 160 mg neratinib/50 mg temsirolimus. Responses were noted in patients with HER2-amplified breast cancer resistant to trastuzumab, HER2-mutant non–small-cell lung cancer, and tumor types without identified mutations in the HER/PI3K/mTOR pathway. |

| Drug studies | Monotherapy or combination therapy | Target | Published year | First author | No. of patients | Type of study | Patient population | Dosage | Patient outcome (primary outcome measures) | Response |
|---|---|---|---|---|---|---|---|---|---|---|
| Neratinib | Combination | Pan-EGFR | 2010 | Burstein | 136 | Phase II | Advanced HER2-positive breast cancer with or without prior trastuzumab treatment | Neratinib: 240–320 mg, dose escalation | Primary end point: 16-week PFS rate for the evaluable population | With ($n$ = 63) vs. without ($n$ = 64) prior trastuzumab, 16-week PFS: 59% vs. 78%, respectively. Median PFS: 22.3 and 39.6 weeks, respectively. ORR: 30% and 13%, respectively. |
| Neratinib | Mono | Pan-EGFR | 2012 | Ito | 21 | Phase I | Advanced solid tumors | Neratinib: 80, 160, 240, or 320 mg orally, dose escalation | Safety, tolerability, MTD, antitumor activity, and PK of neratinib | MTD, RP2D: 240 mg once daily |
| Neratinib | Mono | Pan-EGFR | 2009 | Wong | | Phase I | Advanced solid tumors | Neratinib: 40, 80, 120, 180, 240, 320, 400, and 500 mg, once daily, dose escalation | DLT, MTD, PK, preliminary antitumor activity | MTD: 320 mg once daily orally. PR: 32% (8 of 25 breast cancer patients) |
| Vandetanib | Combination | EGFR, VEGFR | 2014 | Clemons | 126 | Phase II | Postmenopausal with bone-predominant, hormone receptor-positive MBC | Experimental: fulvestrant (500 mg IM) + vandetanib (100 mg tablets; 1 tablet daily until disease progression or intolerance). Placebo comparator: fulvestrant + placebo (100 mg tablets; 1 tablet daily for duration of study). | Significant change in N-telopeptide level, defined as a ≥30% reduction in urinary N-telopeptide level from baseline. | Urinary N-telopeptide response occurred in 66% for fulvestrant plus vandetanib group and 54% for fulvestrant plus placebo group ($p$ = .21). |
| Vandetanib | Mono | EGFR, VEGFR | 2005 | Miller | 44 | Phase II | MBC | Patients were enrolled sequentially into one of two dose cohorts, 100 or 300 mg orally once daily; 28 days defined one cycle. | ORR, PK, and serial pharmacodynamic studies. | There were no objective responses; 1 patient in the 300 mg cohort had stable disease ≥24 weeks. All patients in the 300 mg cohort and 90% of patients in the 100 mg cohort achieved steady-state concentrations exceeding the $IC_{50}$ for VEGF inhibition in preclinical models. |
| Vandetanib | Combination | EGFR, VEGFR | 2012 | Boer | 64 | Phase II | Second-line treatment for advanced breast cancer | Experimental: docetaxel (100 mg/m$^2$ IV every 21 days) + vandetanib (100 mg orally). Placebo comparator: docetaxel (100 mg/m$^2$ IV every 21 days) + placebo. | Number of patients with a disease progression event | Experienced a progression event: vandetanib group (24 [69%]) compared with the placebo group (18 [62%]); HR = 1.19, two-sided 80% CI: 0.79–1.81; two-sided $p$ = .59. |

MBC: metastatic breast cancer; CI: confidence interval; mAb: monoclonal antibody; RR: response rate; ORR: overall RR; PFS: progression-free survival; OS: overall survival; DLT: dose-limiting toxicity; MTD: maximum tolerated dose; CR: complete response; PR: partial response; pCR: pathological complete response; AUC: area under the curve; RP2D: recommended Phase II dose; CBR: clinical benefit rate; HR: hazard ratio; IM: intramuscular injection; IV: intravenous; SD: stable disease; ER: estrogen receptor; VEGFR: vascular endothelial growth factor receptor; PK: pharmacokinetics; CTC: circulating tumor cells; NICE: national institute for health and care excellence; PD: progressive disease; MAPK: mitogen-activated protein kinase; IC50: inhibitory concentration for 50% reduction in binding.