Review Article
Efficacy and mechanism of action of the tyrosine kinase inhibitors gefitinib, lapatinib and neratinib in the treatment of HER2-positive breast cancer: preclinical and clinical evidence

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Abstract: An increasing number of tumors, including breast cancer, overexpress proteins of the epidermal growth factor receptor (EGFR) family. The interaction between family members activates signaling pathways that promote tumor progression and resistance to treatment. Human epidermal growth factor receptor type II (HER2) positive breast cancer represents a clinical challenge for current therapy. It has motivated the development of novel and more effective therapeutic EGFR family target drugs, such as tyrosine kinase inhibitors (TKIs). This review focuses on the effects of three TKIs mostly studied in HER2-positive breast cancer, lapatinib, gefitinib and neratinib. Herein, we discuss the mechanism of action, therapeutic advantages and clinical applications of these TKIs. To date, TKIs seem to be promising therapeutic agents for the treatment of HER2-overexpressing breast tumors, either as monotherapy or combined with other pharmacological agents.

Keywords: HER2-positive breast cancer, tyrosine kinase inhibitors, lapatinib, gefitinib, neratinib, epidermal growth factor receptor family

Introduction
Breast cancer is the most common malignancy in women and is a major public health issue [1, 2]. In the clinic, breast cancer is mainly classified into four molecular subtypes, luminal A/B, human epidermal growth factor receptor type II (HER2) and basal-like [3, 4]. The subtype HER2 represents 25-30% of all breast cancer cases and HER2 overexpression is strongly associated with aggressive phenotype and poor outcomes [5, 6]. The current and approved therapy for this type of cancer is trastuzumab, a humanized monoclonal antibody that binds to the extracellular domain of HER2 [7, 8]. However, de novo or acquired resistance to therapy occurs in some patients [9]. Consequently, new targeted therapies are in development, such as tyrosine kinase inhibitors (TKIs) [10]. This paper aims to integrate knowledge of the signaling pathways associated to the major TKIs proved on HER2-positive breast cancer, lapatinib, gefitinib and neratinib. Moreover, we discuss molecular mechanisms, resistance and clinical trials for each drug, as well as their beneficial therapeutic effects and undesirable side effects.

EGFR family
The EGFR family comprises four distinct membrane tyrosine kinase receptors; EGFR/ErbB-1, HER2/ErbB-2, HER3/ErbB-3 and HER4/ErbB-4 which are activated upon ligand binding to the extracellular domain of these receptors. Afterwards, the formation of receptor homo- or hetero-dimers is induced resulting in phosphorylation of tyrosine kinases residues and cross-
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**Figure 1.** Schematic representation of the action of three TKIs and their interaction with receptors of the EGFR family. As TKIs are homologous to ATP, they compete for the ATP-binding domain of protein kinases preventing their phosphorylation and subsequent activation of the signal transduction pathways, leading to apoptosis, decreased cellular proliferation and eventually cell cycle arrest. Inhibition of phosphorylation of the receptors by TKIs (X); disrupted heterodimer formation by gefitinib, avoid the interaction between receptors (X); upregulated (↑); downregulated (↓).

Phosphorylation, that triggers numerous signaling pathways such as phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK1/2), signal transducer and activator of transcription (STAT), phospholipase C (PLCγ), and/or the modulation of calcium channels [11], This sequence of events induces cellular responses which include proliferation, differentiation and inhibition of apoptosis, giving rise to diseases such as cancer [12].

In a wide range of epithelial cells, including breast, colon, head, neck, kidney, lung, pancreas, and prostate, the overexpression and constitutive activation of the EGFR family members, particularly EGFR and HER2, may trigger cancer initiation, metastasis, and tumor progression [13-15].

In particular, HER2 is overexpressed/amplified in 20-30% of patients with metastatic breast cancer [16]. Moreover, there is a growing evidence that heterodimer formation between receptors of EGFR members resulted in adverse response to therapy [17]. In order to block EGFRs intracellular signaling pathways in breast cancer, the development of novel therapies which include the use of TKIs is currently underway.

**Tyrosine kinase inhibitors**

The TKIs are oral non-peptide anilinoquinazoline compounds homologous of the adenosine triphosphate (ATP). This similarity allows them to compete for the ATP-binding domain of protein kinases preventing phosphorylation and subsequent activation of the signal transduction pathways, leading to apoptosis and decreasing cellular proliferation [18]. Moreover, TKIs target other kinase receptors due to the homology that they share with the EGFR family in the catalytic domain [19] which is highly con-
served across the kinome [20]. Whereby, the actions of TKIs in several kinases cause different effects in the therapeutic use [21, 22].

The main characteristics, mechanisms of action, causes of resistance and clinical evidences of the major TKIs proved on HER2-positive breast cancer, lapatinib, gefitinib and neratinib, are described below.

Lapatinib

Lapatinib is a reversible dual TKI that selectively targets and inhibits HER2 and EGFR with proven effectiveness in clinical trials. This inhibitor has been approved by the US Food and Drug Administration (FDA) since 2007 for metastatic HER2-positive breast cancer treatment. It is commonly used in combination with the chemotherapeutic agent capecitabine on the treatment of breast cancer patients previously treated with trastuzumab, anthracyclines and taxane [23-25]. Moreover, the compound combined with letrozole, an aromatase inhibitor, has been accepted as first-line therapy for metastatic breast cancer that coexpresses hormone receptors and HER2 [26].

Mechanism of action of lapatinib: preclinical evidence

Lapatinib inhibits proliferation of several human cancer cell lines from vulva, breast, lung, and esophagus [27-29]. In vitro studies showed that lapatinib inhibited the activation of the three main EGFR and HER2 downstream signaling pathways, MAPK, PI3K-AKT and PLCγ1, through decreased phosphorylation of target receptors and the Raf, ERK, AKT, and PLCγ1 proteins. Additionally, this TKI increased p38 expression, a stress-induced member of the MAPK pathway that is involved in apoptosis, the subG1 phase of the cell cycle (a hallmark of apoptosis), and the cyclin-dependent kinase inhibitors p21 and p27 [30-32]. Lapatinib inhibited cell proliferation and migration of breast cancer cell lines expressing different levels of EGFR and HER2; however, cells overexpressing HER2 were more sensitive to this TKI [30]. Also, lapatinib increased the pro-apoptotic protein BIM at the transcriptional level and reduced protein expression of survivin, an apoptosis inhibitor protein, which is express in 90% of all breast cancer cases and is cause of poor outcome for this pathology [33-35]. Although lapatinib is a dual TKI that targets both HER2 and EGFR, it has been demonstrated that it also inhibited phosphorylation of HER3 [36]. A resume of lapatinib mechanisms is found in Figure 1.

There is a high incidence of brain metastases in patients with HER2-overexpressing breast cancer even if they were treated with trastuzumab [37, 38]. Interestingly, in a preclinical mouse model, lapatinib could prevent the metastatic outgrowth of HER2-overexpressing breast cancer cells in the brain. In this in vivo metastasis model, lapatinib reduced the phosphorylation of HER2 but it did not affect EGFR, contrary to in vitro studies [30]. Moreover, EGFR small-interfering RNA (siRNA) knockdown in HER2-positive breast cancer cells did not affect the antiproliferative activity of lapatinib, whereas depletion of HER2 causes lapatinib resistance, indicating that lapatinib effects are mediated mainly through HER2 pathway [32]. The stated above suggests a direct correlation between lapatinib sensitivity and HER2 expression only.

A subgroup of HER2-overexpressing tumors also express p95HER2, an amino terminally truncated receptor, that has kinase activity but lacks the epitope recognized by trastuzumab; hence, expression of this form confers resistance to trastuzumab [39]. In addition, p95-HER2 has been considered as a biomarker of an aggressive subtype of HER2-positive breast cancer [40]. Lapatinib inhibited p95HER2, AKT, MAPK phosphorylation and the growth of cells that express the truncate receptors. Moreover, lapatinib showed antitumor activity in p95HER2 tumor xenografts [41].

Other study demonstrated that lapatinib inhibited insulin-like growth factor I (IGF-I) signaling in both trastuzumab-sensitive and -resistant HER2 overexpressing cells [42]. Cross-talk between the IGF-1 receptor and HER2 in trastuzumab-resistant cells increased HER2 receptor phosphorylation [43]. Significantly, lapatinib blocked HER2 and IGF-1R crosstalk [42]. In addition, this compound also increased fragmentation of poly ADP-ribose polymerase (PARP), a protein involved in programmed cell death, and downregulated survivin expression in trastuzumab sensitive and resistant HER2 overexpressing cells [42].
## Table 1. Clinical evidence with lapatinib in HER2-positive breast cancer

<table>
<thead>
<tr>
<th>Therapy [Ref.]</th>
<th>Study type</th>
<th>Patient population (n)</th>
<th>Principal findings</th>
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<tbody>
<tr>
<td>Lapatinib [53]</td>
<td>Phase II, open-label, two-stage, two-cohorts multicenter study.</td>
<td>Advanced or metastatic IBC, HER2 and/or EGFR positive, refractory or recurrent after treatment with an anthracycline. Cohort A: HER2-positive (30). Cohort B: EGFR-positive and HER2 negative (15).</td>
<td>Lapatinib (1500 mg/day) was well tolerated in both cohorts. It showed clinical activity (CR=7%, PR=43%, OR=16.9%, PFS=14 weeks) in heavily pretreated HER2-positive but not in EGFR-positive/HER2-negative patients (PR=6.6%, PFS=4 weeks). The most common AEs in both cohorts included grade 1/2 diarrhea, musculoskeletal pain, and rash. Serious AEs included musculoskeletal pain, dyspnea, and diarrhea. PTEN status does not preclude response to lapatinib. Coexpression of phospho HER2 and phospho HER3 in tumors seems to predict for a favorable response to lapatinib.</td>
</tr>
<tr>
<td>Lapatinib [163]</td>
<td>Phase II, open-label, single-group, multicenter study.</td>
<td>Advanced or MBC with HER2 overexpression, with disease progression during TZ therapy (78).</td>
<td>Lapatinib demonstrated clinical activity (CBR=11.5%, TTP and PFS=15.3 weeks). Doses of 1250 and 1500 mg/day were well tolerated. AEs were rash, diarrhea, nausea, and fatigue with no grade 4 events.</td>
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<tr>
<td>Lapatinib [54]</td>
<td>Phase II, open-label, single-arm, two-cohorts multicenter study.</td>
<td>HER2-positive (cohort A=140) and negative (cohort B=89) advanced MBC prior anthracycline and/or taxane treatment.</td>
<td>Lapatinib dose of 1500 mg/day provided modest clinical benefit (CBR=5.7%) only in HER2-positive patients. PFS after 4 months (34 vs 18%) and OS (29.4 vs 18.6 weeks) were higher in HER2-positive than in HER2-negative patients. The most common AEs were diarrhea, nausea and rash most grade 1/2 with maximum severity of grade 3 in both cohorts. Serious AEs were diarrhea, dehydration, nausea, and vomiting. There were 4 fatal AEs.</td>
</tr>
<tr>
<td>Lapatinib [164]</td>
<td>Phase II, open-label, multicenter. Study was originally designed with two cohorts but cohort B was closed.</td>
<td>Relapsed or refractory invasive, IBC with HER2 overexpression previously treated with anthracycline and taxane plus TZ. (Cohort A=126)</td>
<td>Lapatinib dose of 1500 mg/day was considered potentially clinically effective (ORR=40-15% depending on criteria, PFS=14.6 weeks, OS=8.4 months) in heavily pretreated patients. Likelihood of response to lapatinib was not affected by previous treatment with TZ. The most common serious AEs were dyspnea and pleural effusion. Fatal AEs were possibly treatment related.</td>
</tr>
<tr>
<td>Lapatinib [55]</td>
<td>Phase II, open-label, two-cohorts multicenter studies.</td>
<td>Japanese patients with advanced or MBC with HER2 positive (cohort A=122) and negative (cohort B=22), with disease progression during previously therapy with anthracycline and taxane plus TZ.</td>
<td>Lapatinib dose of 1500 mg/day was well tolerated in both cohorts and effective only in HER2-positive patients. CBR (25 vs 4.5%) and OS (58.3 vs 40 weeks) were higher in cohort A than in cohort B. The most common AE was diarrhea grade 1/2. Patients with tumours harbouring an H1047R PIK3CA mutation or low expression of PTEN derived clinical benefit from lapatinib.</td>
</tr>
<tr>
<td>Lapatinib [165]</td>
<td>Phase II, randomized open-label, parallel-group, multicenter study.</td>
<td>HER2-positive advanced or MBC. Could be previously treated. (69/69).</td>
<td>Lapatinib doses of 1500 mg once daily or 500 mg twice daily were safe and effective without significant differences in clinical activity or the AEs profile between them (ORR=22 and 26%, CBR=29 and 33%, PFS at 4 months=60 and 67%). The most common AEs were diarrhea, rash, pruritus, and nausea, grade 1/2.</td>
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HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

Lapatinib [56] Phase II HER2-positive breast cancer with brain metastases prior TZ treatment. (39).
Lapatinib doses of 750 mg twice daily provided clinical benefit measured as absence of progression in brain metastases (ORR=2.6%, SD≥16 weeks=15.4%). The most common AEs were diarrhea and fatigue grade 2/3.

Lapatinib [166] Phase I single institution study.
HER2-overexpressing breast cancer patients without any restrictions on prior therapies including TZ or lapatinib. (34).
This study used a 3+3 dose escalation design with a starting dose cohort of 1750 mg twice daily, ending with 7000 mg twice daily. The protocol had an amendment consisted in the use of exposure enhancement strategies such as take the medication with food, inhibition of CYP3A4 with ketoconazole and dose fractionation (four times a day) using a dose of 3000 mg. The majority of AEs were grade 1/2 and diarrhea was the most common. Lapatinib dose was escalated to 7000 mg per day with no dose limiting toxicity; however, plasma lapatinib concentrations plateaued in this dose range. 6 patients achieved a response and dramatic responses were seen in 3 patients with lapatinib concentrations approaching 10000 ng/mL.

Lapatinib [167] Phase III, randomized double blind, placebo-controlled multicenter trial.
Patients in early stages of HER2-positive invasive breast prior adjuvant chemotherapy but not TZ. (1571/1576).
Lapatinib (1500 mg) and placebo were administrated daily for 12 months. A review of HER2 status showed that only 79% of the women were HER2-positive. In this group, with a median follow-up of 47.4 months in the lapatinib subgroup and 48.3 in the placebo group disease-free survival events occurred in 13% in the lapatinib group and 17% in the placebo group. OS=6 and 7% in lapatinib and placebo groups respectively. 6% serious AEs occurred in patients taking lapatinib and 5% in patients taking placebo with higher incidences of grade 3/4 diarrhea (6 vs 1%), rash (5 vs <1%), and hepatobiliary disorders (2 vs <1%).

Postmenopausal women with hormone receptor-positive, HER2-positive MBC. (642/644).
Combination therapy (lapatinib 1500 mg/day plus letrozole 2.5 mg/day) was superior to endocrine therapy alone (letrozole 2.5 mg/day). Respectively: PFS=8.2 vs 3.0 months, CBR=48 vs 29%. Diarrhea and rash grade 3/4 were more common in the combination arm versus monotherapy arm. 3 fatal AEs events were possibly treatment related, 1 in combination therapy, 2 in monotherapy.

Lapatinib+vinorelbine [168] Phase I dose-escalation multicenter study.
HER2-positive, advanced or MBC, prior TZ treatment. (30).
The maximal tolerated dose recommended for combination was 1000 mg/day of lapatinib and 22.5 mg/m² of vinorelbine. AEs grade 1/2 were diarrhea, rash and liver enzymes elevation. AEs grade 3/4 were neutropenia, anemia, diarrhea and asthenia.

Lapatinib+capecitabine [23] Phase III randomized two-arms, open label study.
HER2-positive locally advanced or MBC progressed after treatment with, but were not limited to, anthracycline, taxane, and TZ. (163/161).
Combination therapy (lapatinib 1250 mg/day plus capecitabine 2000 mg/m²/day) was superior to capecitabine monotherapy (2500 mg/m²/day). Respectively: TTP=8.4 vs 4.4 months, PFS=8.4 vs 4.1 months, ORR=22 vs 14%, CBR=44-29%. This combination was not associated with an increase in serious toxic effects. The most common AEs were diarrhea, PPE, nausea, vomiting, fatigue and rash. Grade 4 diarrhea occurred in combination therapy arm. One death related to drug toxicity in monotherapy arm.
HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

<table>
<thead>
<tr>
<th>Study Description</th>
<th>Phase/Design</th>
<th>HER2-positive locally advanced or MBC progressed after treatment with, but were not limited to, anthracycline, taxane, and TZ.</th>
<th>Combination therapy (lapatinib 1250 mg/day plus capecitabine 2000 mg/m²/day) showed to be superior versus capecitabine monotherapy (2500 mg/m²/day). Respectively: OS=75.0 vs 64.7%, TTP=27.1 vs 18.6 months, CBR=29.3 vs 17.4%. The most common AE was diarrhea in both arms.</th>
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<tr>
<td>Lapatinib+ capecitabine [169, 170]</td>
<td>Phase III randomized two-arms, open label study.</td>
<td>HER2-positive locally advanced or MBC progressed after treatment with, but were not limited to, anthracycline, taxane, and TZ.</td>
<td>(198/201). \nValues favored combination therapy vs monotherapy. Respectively: ORR=24 vs 14% and TTP=33.3 vs 22.9 months.</td>
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<tr>
<td>Lapatinib+ capecitabine [171]</td>
<td>Phase III randomized two-arms, open label study.</td>
<td>HER2-positive locally advanced or MBC progressed after treatment with, but were not limited to, anthracycline, taxane, and TZ.</td>
<td>(198/201). \nThis combination regimen (capecitabine 2000 mg/twice day for 7 days followed by a 7-day rest and lapatinib 1250 mg/day) provided clinical benefit (CBR=50%, PFS=9.4 months), was well tolerated and reduced gastrointestinal toxicity compared with standard regimen.</td>
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<td>Lapatinib+ capecitabine [172]</td>
<td>Phase II non-randomized open-label, single-center study.</td>
<td>HER2-positive MBC progressed with TZ. (22).</td>
<td>Combination therapy (lapatinib 1250 mg/day plus capecitabine 2000 mg/m²/day) showed to be active in terms of objective CNS response (OR=65.9%). The most common AE were diarrhea, PPE, nausea, fatigue, rash and bilirubin increase. AEs grade 3/4 were diarrhea, PPE and fatigue. The combination therapy could change the management of selected patients with brain metastases, allowing delay to WBRT and its AEs.</td>
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<tr>
<td>Lapatinib+ capecitabine [57]</td>
<td>Phase II open-label, multicenter study.</td>
<td>HER2-positive breast cancer with brain metastases. Previous treatment was allowed except with lapatinib or capecitabine and WBRT. (44).</td>
<td>Lapatinib alone (750 mg/twice day) showed a discrete CNS clinical activity (OR=6%, PFS=2.4 months). In extension phase, combination therapy (lapatinib 1250 mg/day plus capecitabine 1000 mg/m²/day) showed to have clinical activity (OR=20%, PFS=3.7 months). Volumetric reduction in CNS lesions was similar in both therapies. The most common AEs were PPE, diarrhea and nausea. AEs grade 3 were PPE, nausea and diarrhea.</td>
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<tr>
<td>Lapatinib In extension phase: lapatinib+ capecitabine [59]</td>
<td>Phase II open-label, two-cohorts, multicenter study.</td>
<td>HER2-positive breast cancer with brain metastases prior T2 and WBRT treatment.</td>
<td>Combination therapy (lapatinib 1250 mg/day plus capecitabine 1000 mg/m²/twice day) showed to be safe and to offer clinical benefit to this population. Patients treated with the combination and no prior capecitabine had higher OS and PFS than patients with prior capecitabine. (41.7 and 23.9 weeks vs 36 and 18.4 weeks respectively). The most common AEs were diarrhea, vomiting, and nausea and were mainly grade 3 or higher.</td>
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<td>Lapatinib+ capecitabine [173]</td>
<td>Single-arm open-label trial.</td>
<td>HER2 locally advanced or MBC that had progressed following treatment with an anthracycline, a taxane, and TZ alone or in combination. (4283).</td>
<td>Combination therapy (lapatinib 1250 mg/day plus capecitabine 1000 mg/m²/twice day) showed to be safe and to offer clinical benefit to this population. Patients treated with the combination and no prior capecitabine had higher OS and PFS than patients with prior capecitabine. (41.7 and 23.9 weeks vs 36 and 18.4 weeks respectively). The most common AEs were diarrhea, vomiting, and nausea and were mainly grade 3 or higher.</td>
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### HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

<table>
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<th>Study Design</th>
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<th>Outcomes</th>
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<tr>
<td>Lapatinib+capecitabine [174]</td>
<td>Open-label multicenter study.</td>
<td>Japanese patients with HER2-positive invasive and MBC previously treated with anthracyclines, taxanes and TZ. (51).</td>
<td>Combination therapy (lapatinib 1250 mg/day plus capecitabine 1000 mg/m²/twice daily) was well tolerated and showed clinical activity (CBR=50%, TTP=36 weeks). The most common AEs were PPE, diarrhea and stomatitis grade 1/2.</td>
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<tr>
<td>Lapatinib+capecitabine [175]</td>
<td>Single arm open-label trial.</td>
<td>Chinese patients with HER2-positive invasive and MBC prior therapy with a taxane and/or an anthracycline and could have received prior TZ. (52).</td>
<td>Combination therapy (lapatinib 1250 mg/day plus capecitabine 2000 mg/m²/day) showed to be well tolerated and to offer clinical benefit (CBR=57.7%, PFS=6.34 months) to heavily pretreated patients. The most common AEs were PPE, diarrhea, rash, hyperbilirubinemia, and fatigue, all grade 1/2. Grade 3/4 AEs were rash, hyperbilirubinemia, fatigue and neutropenia. PIK3CA mutation status was not associated with CBR nor PFS.</td>
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<tr>
<td>Lapatinib+capecitabine [176]</td>
<td>Phase I pos hoc analysis.</td>
<td>HER2-positive advanced or MBC patients. (38).</td>
<td>Treatment schedule consisted of lapatinib 1250 mg daily, 1 hour before or after breakfast, administered as single agent for the first 10 days, then continuously, in combination with capecitabine 2000 mg/m², starting on day 11 (for the first cycle), and then from day 8, for 14 days out of a 21-day cycle. Cholestyramine was administered twice a day on a continuous basis, long after capecitabine and lapatinib intake. Diarrhea was the main AE: 13.2% grade 1, 10.5% grade 2, 2.6% grade 3 and no grade 4 events. ORR=34.2%, CB=55.3%, PFS=10 months. OS (1 year)=71.2%. The results are comparable with previous reports of conventional administration of the lapatinib-capecitabine regimen and led to a significant reduction in the incidence and severity of diarrhea.</td>
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<td>Lapatinib+Pegylated liposomal doxorubicin [177]</td>
<td>Phase II, open-label, single-arm multicenter study.</td>
<td>HER2-positive with locally advanced, inoperable or MBC with disease progression after TZ therapy. (24).</td>
<td>Treatment consisted in 1250 mg lapatinib daily until progression plus 40 mg/m² of pegylated liposomal doxorubicin at every 4 weeks for a maximum of 6 cycles. This combination showed to be active and safe in HER2-positive MBC, especially suitable for patients with cardiological risk or brain metastases. ORR was 54%, PFS and OS were 5.8 and 23.3 months respectively. The one-year PFS rate was 27% and 1-year OS rate 76%. The most commonly observed AEs were diarrhea, rash and infection. No grade 4 events reported.</td>
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<tr>
<td>Lapatinib+capecitabine vs lapatinib+topotecan [58]</td>
<td>Phase II, open-label, two-arms randomized study.</td>
<td>HER2 breast cancer with brain metastases despite prior standard treatment with WBRT and/or stereotactic radiosurgery and TZ. (13/9).</td>
<td>No CNS-OR was observed with lapatinib (1250 mg/day) plus topotecan (3.2 mg/m²) therapy and showed excessive toxicity (one death possible related to it). ORR=38% in the lapatinib plus capecitabine arm.</td>
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<tr>
<td>Lapatinib+capecitabine vs trastuzumab+capecitabine [178]</td>
<td>Prospective non-randomized two-arms controlled study.</td>
<td>Chinese patients with HER2 invasive and MBC resistant to TZ and previously received taxane therapy. (60/60).</td>
<td>Lapatinib (1250 mg/day) plus capecitabine (2000 mg/m²/day) therapy was superior than TZ therapy (6 mg/kg every 21 days [after the initial 8 mg/kg loading dose]) plus capecitabine (2000 mg/m²/day) for PFS and CBR (6.0 vs 4.5 months, 48 vs 63% respectively).</td>
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**HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib**

**Lapatinib+vinorelbine vs lapatinib+capecitabine** [179]  
A phase II randomized multicenter study.  
HER2-positive MBC patients who had received no more than one chemotherapeutic regimen.  
Arm A: lapatinib plus vinorelbine.  
Arm B: lapatinib plus capecitabine. (70/35).  
Both therapies showed similar outcomes. Combination therapies doses were: Arm A, 1250 mg/day lapatinib plus 20 mg/m²/day vinorelbine. Arm B, 1250 mg/day lapatinib plus 2000 mg/m²/day capecitabine. PFS for both therapies=6.2 months, OS=24.3 for arm A vs 19.4 months for arm B. 42 patients opted to cross over and second evaluation was made: PFS=3.25 months for lapatinib plus vinorelbine vs 4.0 months lapatinib plus capecitabine. The most commonly observed AEs were diarrhea, neutropenia, PPE, rash, nausea, and fatigue. There were more serious AEs in arm A than in arm B.

**Lapatinib+pazopanib** [180]  
Phase II two-cohorts multicenter study.  
HER2 relapsed or refractory IBC.  
Cohort 1: previous history of IBC and documented recurrence in the skin and/or other disease sites by radiologic assessments (76).  
Cohort 2: cutaneous disease documented with photographs (88).  
Cohort 1: combination therapy (lapatinib 1500 mg/day plus pazopanib 800 mg/day) had higher clinical activity than monotherapy (lapatinib 1500 mg/day) for ORR (29 vs 45%) respectively, but was not well tolerated.  
Cohort 2: combination therapy (lapatinib 1000 mg/day plus pazopanib 400 mg/day) was well tolerated and was superior to monotherapy (lapatinib 1500 mg/day) for CBR (58 vs 47%) but it was not for PFS. Combination showed an increase in toxicity.

**Lapatinib+pazopanib** [181]  
Phase II randomized two-cohorts, multicenter study.  
HER2 invasive and MBC. No prior anticancer therapy (except hormonal therapy) was permitted in the randomized.  
Cohort 1: randomized (150).  
Cohort 2: nonrandomized (40).  
Combination therapy in cohort 1 (lapatinib 1000 mg/day plus pazopanib 400 mg/day) was not superior to monotherapy (lapatinib 1500 mg/day) for PDR (36.2 vs 38.9%), but it was for ORR (36.2 vs 22.2%).  
In cohort 2: (lapatinib 1500 mg/day plus pazopanib 800 mg/day) ORR=33.3%. AEs grade 3/4 including diarrhea, hypertension and liver enzymes elevation were higher in combination in cohort 2 than in combination in cohort 1.

**Lapatinib+bevacizumab** [182]  
Phase II open-label, multicenter study.  
HER2 invasive and MBC. (52).  
Lapatinib (1500 mg/day) plus bevacizumab (10 mg/kg every 2 weeks) therapy was active in terms of PFS (24.7 weeks), CBR (30.8%) and ORR (13.3%). The most common AEs were diarrhea, rash, fatigue, nausea, headache, and epistaxis, grade 1/2. AEs grade 3/4 were rash, hypertension, diarrhea, hyperuricemia, hydronephrosis, gastrointestinal hemorrhage and liver enzyme alteration.

**Lapatinib+paclitaxel** [183]  
Phase III randomized two-arms, double-blind, multicenter study.  
Newly diagnosed HER2 MBC. (215/215).  
Lapatinib (1500 mg/day) combined with paclitaxel (80 mg/m² weekly) was superior to paclitaxel alone (80 mg/m² weekly) for OS, PFS and ORR (27.8 vs 20.5 months, 9.7 vs 6.5 months and 69 vs 50% respectively). Incidence of grades 3/4 diarrhea and neutropenia was higher in the lapatinib plus paclitaxel arm.

**Lapatinib+paclitaxel** [184]  
Phase III randomized two-arms, double-blind, multicenter study.  
HER2-negative or initially untested, locally advanced or MBC previously untreated. (291/288).  
Combination therapy (paclitaxel 175 mg/m² every 3 weeks plus lapatinib 1500 mg/day) was superior to monotherapy (paclitaxel 175 mg/m² every 3 weeks) in terms of ORR, CBR, TTP (63.3 vs 37.8%, 69.4 vs 40.5%, 36.4 vs 25.1 weeks, respectively). Rash and diarrhea grade 3 were higher in the combination arm.
### HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

<table>
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<th>Combination</th>
<th>Study Type</th>
<th>Description</th>
<th>Results/Notes</th>
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</thead>
<tbody>
<tr>
<td>Lapatinib+nab-paclitaxel [185]</td>
<td>Phase II open-label, single-arm, multicenter study.</td>
<td>HER2 MBC with no more than one prior chemotherapeutic regimen. (60).</td>
<td>The recommended doses for this combination therapy were lapatinib: 1000 mg and nab-paclitaxel 100 mg/m². This combination showed to offer clinical benefit (ORR=53%, PFS=39.7 weeks, TTP=41 weeks). Data were consistent with those reported for lapatinib in combination with paclitaxel.</td>
</tr>
<tr>
<td>Lapatinib+paclitaxel+gemcitabine [186]</td>
<td>Phase I open-label study.</td>
<td>HER2 early breast cancer, no previously treated. (13).</td>
<td>The recommended doses for this combination were lapatinib 1000 mg/day, paclitaxel 80 mg/m² on days 1 and 8 and gemcitabine 1000 mg/m² on days 1 and 8, every 3 weeks. The most frequent AE was neutropenia grade 3/4. Other AEs of more than grade 2 were rare: liver enzyme alteration, anorexia, gastritis, abdominal pain, diarrhea and paronychia. Combination therapy was well tolerated and showed clinical benefit (PR=61.5%, CR=30.7%).</td>
</tr>
<tr>
<td>Lapatinib+TZ [187]</td>
<td>Phase I dose-escalation study.</td>
<td>HER2 advanced or MBC. (54).</td>
<td>The optimally tolerated regimen was lapatinib 1000 mg/day plus TZ 2 mg/kg weekly (after the initial 4 mg/kg loading dose). Combination therapy showed to offer clinical benefit (ORR=15%). The responders had received prior TZ therapy in combination with cytotoxic chemotherapy. The most frequent AEs were diarrhea, rash, fatigue and nausea. Most common grade 3 events included diarrhea, fatigue, and rash.</td>
</tr>
<tr>
<td>Lapatinib+TZ [24]</td>
<td>Phase III randomized two-arms open-label multicenter study.</td>
<td>HER2 MBC progressed in the last TZ treatment. Must have received prior anthracycline- and taxane-based regimens. (148/148).</td>
<td>Combination therapy (lapatinib 1000 mg/day plus TZ 2 mg/kg weekly [after the initial 4 mg/kg loading dose]) was superior to lapatinib alone (1500 mg/day) for PFS and CBR (12.0 vs 8.1 weeks and 24.7 vs 12.4% respectively). Incidence of diarrhea was higher with the combination arm.</td>
</tr>
<tr>
<td>Lapatinib+TZ [188]</td>
<td>Phase II single-arm, multicenter study.</td>
<td>HER2 invasive breast cancer. (66).</td>
<td>Combination therapy (TZ [4 mg/kg loading, then 2 mg/kg/week] and lapatinib 1000 mg/day) showed to be clinically active (CR=27%, pRR=22%). Only 3% of the patients experienced disease progression. The most common AEs were diarrhea, rash, fatigue and nausea, all grade 1/2. AEs grade 3/4 were liver enzymes alterations, diarrhea, rash and hypertension.</td>
</tr>
<tr>
<td>Lapatinib vs TZ [189]</td>
<td>Phase II randomized open-label study.</td>
<td>HER2 positive MBC not previously treated with chemotherapy and/or anti-HER2 agents for metastatic disease. (19).</td>
<td>No significant difference in terms of response at 8 weeks was observed according to treatment arm (75 and 67% MR or more with lapatinib [1500 mg daily for 8 weeks] and TZ [8 mg/kg on loading dose, followed by weekly trastuzumab at the dose of 2 mg/kg] respectively). In the overall population, persistence in protocol, PSF and OS were 3.8, 7.3 and 43 months, respectively. MBC patients bearing tumors with markers such as “HER2-enriched” subtype and/or having high HER2/p95HER2 protein expression ratio are exquisitely sensitive to anti-HER2 agents and could be candidates for studies aimed at establishing chemotherapy-free regimens.</td>
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# HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study Design</th>
<th>Study Duration</th>
<th>HER2 Subgroup</th>
<th>Result Information</th>
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<tbody>
<tr>
<td>Lapatinib+TZ+docetaxel [190]</td>
<td>Phase I open-label, dose-escalation,</td>
<td>HER2 MBC. (53).</td>
<td>75 mg/m² every three weeks and lapatinib 1000 mg plus docetaxel 100 mg/m² every three weeks, both with weekly TZ (loading dose of 4 mg/kg followed by a fixed dose of 2 mg/kg) and including use of prophylactic granulocyte colony stimulating factor. Combination therapy showed to offer clinical benefit (ORR=64%). The most common AEs were diarrhea and nausea. Grades 3/4 were neutropenia, diarrhea, leukopenia, peripheral neuropathy, and rash.</td>
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<tr>
<td>Lapatinib+TZ+paclitaxel [191, 192]</td>
<td>Phase III randomized open-label,</td>
<td>HER2 invasive early breast cancer. (152/154).</td>
<td>Combination therapy (lapatinib 1000 mg/day plus TZ 2 mg/kg weekly [after the initial 4 mg/kg loading dose] plus paclitaxel 80 mg/m²/week) was superior to TZ alone (2 mg/kg weekly [after the initial 4 mg/kg loading dose] and to lapatinib alone (1500 mg/day) for CR (51.3 vs 29.5 vs 24.7% respectively). Grade 3 diarrhea and liver enzyme alteration were higher in the combination arm. 3 years EFS was 78% for lapatinib, 76% for TZ and 84% for combination. 3 years OS was 93% for lapatinib, 90% for TZ and 95% for combination. EFS and OS did not differ between treatment groups but patients who achieve CR after neoadjuvant anti-HER2 therapy have longer EFS and OS than do patients without CR.</td>
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<tr>
<td>Lapatinib+TZ+paclitaxel [193]</td>
<td>Phase II single-arm, multicenter study.</td>
<td>HER2 surgically resected invasive breast cancer. (122).</td>
<td>Lapatinib given concurrently with paclitaxel and TZ is feasible and did not add cardiac toxicity. The dose of lapatinib in this regimen should not exceed 750 mg daily. Diarrhea grade 3/4 was the most common serious AE. An early aggressive management of this AE is recommended.</td>
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<tr>
<td>TZ-emtansine vs capecitabine+lapatinib [194]</td>
<td>Phase III randomized two-arms study.</td>
<td>HER2-positive, unresectable locally advanced or MBC previously treated with TZ and taxane. (495/496).</td>
<td>TZ-emtansine (3.6 mg/kg intravenously every 3 weeks) delayed the time to symptom worsening compared with capecitabine (1000 mg/m² orally twice a day, on days 1 through 14 of a every 3 week cycle) plus lapatinib (1250 mg orally daily). 7.1 vs 4.6 months, respectively. In the TZ-emtansine arm, 55.3% of patients developed clinically significant improvement in symptoms vs 49.4% in the capecitabine plus lapatinib arm. Diarrhea cases were higher in the capecitabine plus lapatinib arm than in the TZ-emtansine arm.</td>
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<tr>
<td>Lapatinib+TZ+chemotherapy [195]</td>
<td>Phase II randomized three-arms</td>
<td>HER2 infiltrating breast cancer. (36/39/46).</td>
<td>Combination of chemotherapy (fluorouracil 600 mg/m², epirubicin 75 mg/m², and cyclophosphamide 600 mg/m² administered every 3 weeks) with lapatinib (1000 mg/day) and TZ (2 mg/kg weekly after the initial 4 mg/kg loading dose) was superior to chemotherapy plus lapatinib and chemotherapy plus TZ for CR (46.7 vs 25.0 vs 26.3% respectively). The majority of the patients required lapatinib dose reduction because high grade 3 diarrhea incidence.</td>
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HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

Chemotherapy+TZ vs chemotherapy+lapatinib [196]

Phase II randomized two arms, open label, multicenter trial.

Stages I, II, III or inflammatory breast cancer HER2 positive. (50/52).

Chemotherapy consisted in epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m² both administered on day 1 every 21 days for four cycles followed by docetaxel 100 mg/m² administered on day 1 every 3 weeks for four cycles. The anti-HER2 therapy was added to docetaxel as follows: patients in arm A received chemotherapy plus TZ 6 mg/kg (after a loading dose of 8 mg kg/1) on day 1 every 21 days (EC-DT). Patients in arm B received chemotherapy plus a daily dose of lapatinib 1250 mg orally (EC-DL). EC-DT exhibited higher efficacy than EC-DL in terms of CR (52.1 vs 25.5% in breast, 47.9 vs 23.5% in breast and axila). Grade 3/4 toxicity rates were similar across arms except for diarrhea, which was more frequent in the EC-DL arm.

Chemotherapy+TZ vs chemotherapy+lapatinib [197]

Phase III randomized two arms, open label, multicenter trial.

Untreated HER2-positive operable or locally advanced breast cancer. (309/311).

Chemotherapy consisted in epirubicin 90 mg/m² plus cyclophosphamide 600 mg/m² both administered every 3 weeks for four cycles followed by docetaxel 100 mg/m² administered on day 1 every 3 weeks for four cycles. The anti-HER2 therapy was added to docetaxel as follows: patients in arm A received chemotherapy plus TZ 6 mg/kg (after a loading dose of 8 mg kg/1) on day 1 every 21 days (ECH-TH). Patients in arm B received chemotherapy plus a daily dose of lapatinib 1000-1250 mg orally (ECH-TL). ECH-TH exhibited higher efficacy in terms of CR (30.3 vs 22.7% respectively) than ECH-TL. Chemotherapy with trastuzumab was associated with more edema (39.1% vs 28.7%) and dyspnea (29.6 vs 21.4%), and ECL-TL with more diarrhea (75.0 vs 47.4%) and skin rash (54.9 vs 31.9%). Less AE’s were reported in the ECH-TH group than in the ECL-TL group.

MBC, metastatic breast cancer; IBC, inflammatory breast cancer; CR, complete response; PR, partial response; OR, objective response; PFS, progression free survival; PTEN, tensin homologue deleted in chromosome 10; AE, adverse event; CBR, clinical benefit rate; TZ, trastuzumab; ORR, objective response rate; OS, overall survival; SD, stable disease; TTP, time to progression; CNS, central nervous system; PPE, palmar-plantar erythrodysesthesia; WBRT, whole brain radiotherapy; PS, performance status; PDR, progressive disease rate; pPR, pathological response rate; EFS, event free survival; MR, minimal response.

Table 2. Clinical evidence with neratinib in HER2 breast cancer

<table>
<thead>
<tr>
<th>Therapy [Ref.]</th>
<th>Study type</th>
<th>Patient population (n)</th>
<th>Principal findings</th>
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<tbody>
<tr>
<td>Neratinib [143]</td>
<td>Phase II open-label, two-cohorts, multicenter study.</td>
<td>HER2 advanced and MBC. Cohort A: Prior TZ treatment (66). Cohort B: No prior TZ treatment (70).</td>
<td>Neratinib (240 mg daily) was well tolerated and had clinical activity (PFS at 16 weeks=59 and 78%, OR=24 and 56% for cohort A and cohort B respectively). The most common AEs were diarrhea, nausea, vomiting, and fatigue. Diarrhea was the most frequent grade 3/4 AE.</td>
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<tr>
<td>Treatment</td>
<td>Study Type</td>
<td>Study Details</td>
<td>Clinical Activity</td>
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<tr>
<td>Neratinib + Paclitaxel</td>
<td>Phase I/II open-label, two-part study.</td>
<td>HER2-positive MBC and prior TZ therapy. Group A: no more than one prior chemotherapy regimen and no prior lapatinib exposure (71). Group B: no more than three prior cytotoxic chemotherapy regimens with prior lapatinib exposure permitted (31).</td>
<td>Combination therapy (neratinib 240 mg daily plus paclitaxel 80 mg/m²) was well tolerated with no unexpected toxicity and had clinical activity in both groups (OR=71 and 77%, PFS=63.1 and 52.1 weeks for group A and B respectively) with a CB=82% for all evaluable patients. The most common AEs were diarrhea, neuropathy, alopecia, nausea, neutropenia, leukopenia and anemia. The most common grade 3/4 AEs were diarrhea, neutropenia, leukopenia and anemia.</td>
</tr>
<tr>
<td>Neratinib + Paclitaxel + TZ</td>
<td>Phase I open label, dose escalation, multicenter study.</td>
<td>HER2-positive MBC previously treated with anti-HER agent(s) and a taxane. (21).</td>
<td>Combination therapy consisted in neratinib (120 up to 240 mg/day) with TZ (4 mg/kg loading dose, then 2 mg/kg weekly) and paclitaxel (80 mg/m² days 1, 8, and 15 of a 28-day cycle). The recommended dose of neratinib for this combination therapy was 200 mg/kg. It showed to have clinical activity (OR=38%, CB=52% and median TTP=3.7 months). Common grade 3/4 AEs were diarrhea (38%), dehydration (14%), electrolyte imbalance (19%), and fatigue (19%).</td>
</tr>
<tr>
<td>Neratinib + Vinorelbine</td>
<td>Phase I/II multicenter, open-label study.</td>
<td>HER2-positive MBC, divided according to prior or no prior lapatinib treatment. (12/56).</td>
<td>Combination therapy (neratinib 240 mg plus vinorelbine 25 mg/m²) was well tolerated with no unexpected toxicity. It showed to have clinical activity (OR=8 vs 41%, PFS=24.0 vs 47.7 weeks, CB=5 vs 39%, prior lapatinib vs no prior lapatinib respectively). The most common AEs were diarrhea, neutropenia, vomiting, nausea and fatigue. The most common grade 3/4 AEs were neutropenia (46%), diarrhea (28%), and leukopenia (17%).</td>
</tr>
<tr>
<td>Neratinib + Capecitabine</td>
<td>Phase I/II multinational open-label study.</td>
<td>HER2-positive MBC or locally advanced breast cancer, previously treated with TZ and taxane. There is a sub-group previously treated with lapatinib. (61/7).</td>
<td>Combination therapy (neratinib 240 mg plus capecitabine 1500 mg/m² daily) showed to have clinical activity (OR=64 vs 57%, PFS=40.3 vs 35.9 weeks, patients with no prior lapatinib exposure vs patients previously treated with lapatinib respectively). The overall most common grade 3/4 AEs were diarrhea (26%), PPE (14%), asthenia (4%) and vomiting (4%).</td>
</tr>
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</table>

MBC, metastatic breast cancer; TZ, trastuzumab; PFS, progression free survival; OR, objective response; AEs, adverse events; CB, clinical benefit; TTP, time to progression; PPE, palmar-plantar erythrodysesthesia.
In addition, lapatinib inhibited activation of nuclear factor κB (NF-κB) in HER2-overexpressing breast cancer cells [44]. The TKI inactivates NF-κB through reducing phosphorylation of its inhibitor IkB-α via blocking the PI3K/AKT cascade [44]. This fact is relevant due to co-operation between HER2 and NF-κB signaling which causes tumor resistance to radiotherapy [45].

Overexpression of EGFR and HER2 contributes to clinical radiation resistance [46] and several EGFR inhibitors sensitize tumor cells to ionizing radiation [46-48]. In this regard, lapatinib treatment enhanced the radiosensitization of EGFR- and HER2-overexpressing breast cancer cells through inhibition of MEK/ERK signaling pathway [49, 50].

In the SK-BR-3 HER2-amplified breast cancer cell line prolonged exposure to lapatinib reduced the expression and activity of the enzyme topoisomerase IIα, which renders cells resistant to the cytotoxic effects of doxorubicin, etoposide, and m-AMSA [51].

Lapatinib regulates several microRNAs (miR) that play an important role in the anti-tumor action in the HER2-positive breast carcinoma cells [52]. In this regard, lapatinib treatment upregulated miR575 and miR-1225-5p expression, contributing in this manner to downregulation of the oncogenic protein phospholipase C PLCXD1 (phosphatidylinositol-specific phospholipase-C-X-domain-containing-1), a target transcript of miR-575 and miR1225-5p [52].

**Lapatinib: clinical evidence and side effects**

Several clinical studies in HER2 breast cancer have evaluated safety, dosing schedules and efficacy of lapatinib as monotherapy or in combination with other therapies. The outcomes depend on population features, tumor type, stage and prior cancer treatments.

In all the studies listed in Table 1, lapatinib and its combinations provided clinical benefit in different grades in HER2 breast cancer patients. In contrast, HER2 negative and EGFR positive cancer patients did not experience benefit with this TKI [53-55].

In general, the addition of lapatinib to another therapy improved the efficacy compared with the drug alone and in some cases without increasing the adverse events profile or its severity.

Some studies have assessed the efficacy of lapatinib alone and in combination with capecitabine in HER2 breast cancer patients with progressive brain metastases. Only modest efficacy has been found with monotherapy while combination therapy has shown to be more effective suggesting that lapatinib plus capecitabine could be an alternative treatment for specific patients to delay whole brain radiotherapy and its adverse effects [Table 1] [56-59].

**Lapatinib resistance and toxicity**

Several cancers generate resistance to TKIs through modifications in different proteins implicated in downstream signaling of their target receptors [60]. Herein, we will revise the proteins and cellular processes known to be involved in acquiring resistance to lapatinib.

Dysregulation of the PI3K pathway is frequent in breast cancer leading to its constitutive activation [61, 62]. Mutations in the catalytic subunit of PI3K (PIK3CA) have been reported in 20-30% of breast cancer patients with HER2 amplification [63, 64]. In HER2 overexpressing cells carrying PIK3CA, the antiproliferative and proapoptotic effects of lapatinib as well as its ability to inhibit survivin and AKT phosphorylation was less pronounced than in those cells without PIK3 mutations [33, 65]. Interestingly, PIK3CA has been considered a biomarker of resistance to the EGFR family targeted therapy [66]. Another abnormal activation of the PI3K pathway is the loss of phosphatase and tensin homologue deleted in chromosome 10 (PTEN) function that has been observed in 20% to 25% of primary breast cancers [63, 67]. In this regard, it has been demonstrated that loss of PTEN expression conferred resistance to lapatinib in both PTEN knockdown cells and tumor xenografts with HER2 overexpression, through maintaining the activation of the AKT signaling pathway [65, 68]. However, another study did not find association between PTEN protein levels and resistance to lapatinib in a panel of 17 HER2-amplified cell lines [69]. This was consistent with two studies that did not show association between PTEN and lapatinib response.
HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib using cell lines and tissues of inflammatory breast cancer patients with PTEN deficiency [53, 70].

Hypoxia promotes lapatinib resistance in HER2-positive breast cancer cells by inducing high levels of hypoxia inducible factor (HIF)-1α and reducing the dual specificity phosphatase2 (DUSP2) expression that leads to activation of the ERK pathway [71].

Autophagy plays an important role in the development of drug resistance in breast cancer cells [72]. Indeed, increase of autophagosome formation was observed in lapatinib resistant breast cancer cell lines. Accordingly, treatment with autophagy inhibitors restored lapatinib sensitivity [73].

Acquired resistance to lapatinib in HER2-positive breast cancer cells can also be mediated by induction of heregulin (HRG) and subsequent formation of HER3-EGFR heterodimers that promote an HRG-HER3-EGFR-PI3K signaling axis [74].

In breast cancer, the overexpression of CD24, a glycosylphosphatidylinositol-anchored membrane protein, is associated with a poor patient prognosis [75]. Noteworthy, CD24 contributes to resistance to lapatinib in HER2-positive breast cancer cells. In fact, knockdown of CD24 downregulated HER2 expression, AKT phosphorylation and increased the sensitivity of cells to lapatinib treatment [76].

Similarly, the overexpression of Neuromedin U (NmU), a neuropeptide that has been associated with poor patient outcome in HER2-overexpressing tumors, is also associated with resistance to lapatinib. NmU knockdown significantly decreased migration, motility, invasion and resistance to anoikis in HER2 overexpressing breast cancer cells [77].

In a HER2-overexpressing/estrogen receptor (ER) positive breast cancer cell line treated continuously with lapatinib, increased ER signaling was observed. This was due to activation of the transcription factor forkhead box protein 03a (FOX03a) in response to ErbB2-PI3K-AKT signaling inhibition, resulting in autoresistance to this inhibitor [78]. Consequently, ER signaling assumed a more prominent role in cell survival during the development of acquired resistance to the TKI. Accordingly, the combination of lapatinib with antagonists of the ER such as tamoxifen or fulvestrant prevented the development of lapatinib resistance [78]. In addition, in tumor biopsies of patients with early-stage breast cancer, after 14 days of lapatinib therapy, increased FOXO3a, progesterone receptor and Bcl-2 expression was observed [78].

In human breast cancer lines and xenograft models it was demonstrated that FAM83A overexpression, an oncogenic protein, is involved in lapatinib resistance [79]. Interestingly, FAM83A downregulation decreased proliferation and rendered cells sensitive to lapatinib. The mechanism by which this protein conferred resistance to lapatinib may include the interaction of FAM38A with cRAF and PI3K, maintaining the activation of MAPK signaling. Indeed, breast cancer patients with high FAM83A expression have poor prognosis [79].

Some receptors with tyrosine kinase activity have been involved in lapatinib resistance. In this sense, the aberrant expression of AXL, recepteur d’origine nantais (RON) and fibroblast growth factor receptor 2 (FGFR2) has been reported to play an important role in lapatinib resistance through eliciting PI3K/AKT and ERK signaling activation. In fact, inhibitors that target AXL, RON and FGFR2 restored lapatinib sensitivity in HER2 breast cancer cell lines [36, 80, 81].

Protein tyrosine kinase 6 (PTK6), Src (both non-receptor tyrosine kinase proteins), the chemokine CXCL12 and its receptor (CXCR4) are involved in breast cancer progression, regulating several cellular processes involved in the malignant phenotype, such as cell proliferation, survival, invasion and migration. Current researches have demonstrated that increased levels of PTK6 and Src expression and phosphorylation respectively, are involved in lapatinib resistance through EGFR-dependent signaling. Further, Src family kinases mRNA expression was upregulated in HER2-positive tumors treated with lapatinib. In fact, treatment with Src inhibitors in combination with lapatinib reduces AKT and ERK1/2 phosphorylation restoring the sensitivity of resistant cells to lapatinib [82-85]. Moreover, lapatinib resistant cells also show high levels of expression of CXCR4. Blockade of this chemokine receptor
HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

with a specific antibody reduced the invasive ability of lapatinib resistant cells. Notably, the combination of CXCR4 antibody and a Src inhibitor saracatinib, resulted in a greater inhibition of the invasiveness compared with either agent alone in lapatinib resistant cells. These findings suggest that blockade of Src and CXCR4 could be utilized as a therapeutic option in patients with acquired resistance to lapatinib [84].

A key feature for acquired lapatinib resistance is the fact that lapatinib treatment in HER2-positive breast cells induces the activation of several networks of kinases that contribute to induce lapatinib resistance. Efforts for dealing with this tumor response include the pharmacological inhibition of the adaptive kinome response. In this regard, inhibitors of epigenetic enzymes of BET family bromodomains suppressed the kinome reprogramming induced by lapatinib [86].

Aberrant miR-630 expression is related with lapatinib resistance in HER2-positive breast cancer cells. Overexpression of this miR reduced the metastatic phenotype and restored the drug sensitivity through decreased IGF1R expression and subsequent inhibition of the phosphorylation of HER2 and EGFR in lapatinib resistant cells [87].

In HER2-positive breast cancer cells, elevated expression of the alpha catalytic subunit of cyclic AMP-activated protein kinase A (PRKACA) confers resistance to lapatinib via increased phosphorylation of the pro-apoptotic molecule BAD [88].

The Kruppel-like transcription factors (KLFs) 4 and 5 (KLF4/5) play an important role in cell proliferation, differentiation, and transformation and have been considered as possible prognostic factors in breast cancer [89, 90]. In fact, a positive correlation between HER-2/neu and KLF5 has been described [90]. In HER2-positive breast cancer cells, lapatinib treatment upregulated KLF4/5 protein expression. In these cells, KLF4 and/or KLF5 depletion restored lapatinib sensitivity and reduced basal mRNA and protein levels of the anti-apoptotic factors myeloid cell leukemia 1 (MCL1) and B-cell lymphoma-extra-large (BCL-XL) [91]. The activation of another transcription factor, the aryl hydrocarbon receptor (AhR), promotes several signaling pathways as EGFR, PI3K/AKT and ERK. In fact, the induction of apoptosis by lapatinib is inhibited by this receptor activated by its external ligand (2,3,7,8-tetrachlorodibenzo-p-dioxin), indicating its participation in lapatinib resistance [92].

In patients with residual breast cancers following administration of taxane/anthracycline-based chemotherapy used concurrently with trastuzumab, loss of HER2 gene amplification has been reported [93]. Interestingly, HER2 breast cancer cells also showed loss of HER2 expression after chronic exposure to lapatinib, conferring resistance to this inhibitor [81]. Another mechanism that limits the antitumor activity of lapatinib is the upregulation of HER3 induced by this TKI, maintaining the activation of AKT and MAPK pathways. These evidences advice the use of HER2/HER3 inhibitors combined in the treatment of HER2-positive breast cancer [94, 95].

Gefitinib

Gefitinib is a reversible EGFR TKI that has been approved by the FDA for the treatment of advanced non-small cell lung carcinoma with activating EGFR mutations [96, 97].

EGFR is overexpressed in breast cancer tissue with a positivity range of 20-70% [98, 99]. Overexpression of this receptor is associated with aggressive metastatic breast tumors. In addition, breast tumors that co-overexpress EGFR and HER2 exhibited a worse outcome than tumors that overexpressed either receptor alone [100]. Interruption of EGFR function with specific TKIs may disrupt EGFR-HER2 cross-talk, resulting in HER2 inactivation [101].

Mechanism of action of gefitinib: preclinical evidence

Gefitinib inhibits the growth of both breast cancer cell lines and human tumor xenografts expressing different levels of EGFR or HER2 [101-103]. Gefitinib effects on HER2 and EGFR coexpressing breast cancer cells are mediated by reducing basal EGFR, HER2 and HER3 phosphorylation, resulting in the blockage of downstream signaling of the AKT, MAPK and STAT3 pathways [101-105]. Also, this TKI increased p27 levels and the subG1/G1 phases of the cell cycle; reduced cyclin D1 and Cdk4. In addition,
gefitinib reduced the phosphorylation of glycogen synthase kinase 3 beta (GSK-3β, a target of the AKT kinase) [101, 106]. In EGFR-HER2 breast cancer cells, gefitinib induced cytostatic and apoptotic effects [103]. This action of gefitinib is in part mediated by increased p38 MAPK levels [102], dephosphorylation of FOXO3a with a subsequent increased of p27Kip1, caspase 3 and BIM protein expression [105, 106]. Gefitinib has also been shown to downregulate the mTOR signaling pathway in human breast cancer cells [107].

In a similar manner as described above in the cell lines, gefitinib inhibited EGFR and MAPK phosphorylation in tumor biopsies. However, gefitinib had no effect on AKT phosphorylation or Ki67 levels. Moreover, the TKI did not increase p27 levels [108].

Gefitinib treatment disrupted the formation of the HER3/HER2 heterodimer and further association of HER3 with p85α the regulator subunit of PI3K [101]. In addition, the TKI inhibited the activation of the EGFR/HER2 and EGFR/HER3 heterodimers mediated by EGF and heregulin, respectively [102].

EGFR overexpression did not determine gefitinib sensitivity in the particular case of HER2 overexpressing breast cancer [103, 104]. In this regard, gefitinib was more potent to inhibit the proliferation of breast cancer cells with high levels of HER2 and low levels EGFR compared to those cells with high levels of EGFR without HER2 expression [101, 102, 104]. In contrast, gefitinib effects on AKT, MAPK, and p27 were not seen in EGFR-negative breast cancer cells [101]. Interestingly, inhibition of MAPK phosphorylation was observed in EGFR-negative tumor biopsies [108], suggesting that gefitinib may be inhibiting other EGFR family members [108].

In the same way as observed with lapatinib, prolonged exposure to gefitinib induced resistance to doxorubicin, etoposide, and m-AMSA through downregulation of topoisomerase IIα [51]. A graphic summary of gefitinib mechanisms of action is found in Figure 1.

Gefitinib: clinical evidence and side effects

There are only two phase I/II clinical studies that assess the effects of different gefitinib-combination therapies specifically in HER2-positive metastatic breast cancer patients. The first one was undertaken in 35 patients divided according to prior systemic therapy. It demonstrated that the combination of 250 mg/day of gefitinib with 2 mg/kg weekly (after the initial 4 mg/kg loading dose) of trastuzumab was safe. However, there was no greater clinical benefit compared to trastuzumab monotherapy. A possible explanation of the lack of clinical activity in this study was that coupling between HER2 and HER3 and activation of the last one would not be completely inhibited by the combination of gefitinib and trastuzumab [109]. The other study included 31 patients treated with 250 mg/day of gefitinib, 6 mg/kg every 3 weeks (after the initial 8 mg/kg loading dose) of trastuzumab and 60 mg/m² every 3 weeks of docetaxel. The values obtained were considered similar to those in other three drug combination regimens including trastuzumab and chemotherapy, with the advantage of less toxicity because the avoidance of a second chemotherapeutic agent. Additionally, no relationship between assessed biomarkers expression and response to therapy was found. In regard to EGFR, 95% of the biopsies showed little or no signal when evaluated by immunohistochemistry, although 47% of this subpopulation responded to the therapy [110].

Gefitinib alone or in combination with other drugs has been assessed in several clinical studies with HER2-negative breast cancer patients. The results are inconsistent but in some cases there is a correlation between the outcome and prior therapies administrated. We discuss these studies below.

In a clinical trial conducted in women with stage IIIb/IV advanced breast cancer resistant to chemotherapy, gefitinib monotherapy showed only a stabilization of disease for at least 3 months, neither partial nor complete response were achieved. Interestingly, a good correlation between the degree of inhibition of EGFR phosphorylation in skin and in breast tumors after gefitinib treatment was reported. Therefore, the inefficacy of this TKI could be explained by the lack of EGFR tumor dependence [108]. In other study, gefitinib was not effective in patients with recurrent or refractory breast cancer previously treated with taxane and anthracycline [111].
HER2-positive breast cancer treatment with lapatinib, gefitinib and neratinib

Moreover, studies in patients with ER and/or progesterone receptor positive breast adenocarcinoma [112, 113], primary untreated breast cancer [114] and metastatic breast cancer [115], it was found that the addition of gefitinib to standard treatments (chemotherapy or hormonal therapy) did not further increase the clinical benefit. However, in some cases it caused higher toxicity and serious adverse events such as dehydration, diarrhea, fatigue, stomatitis, hypokalemia and neutropenia.

In contrast, Polychronis et al., reported that gefitinib alone and in combination with anastrozol was effective for reducing the tumor size in ER-EGFR positive breast cancer naive patients. The most frequently adverse effects presented with both therapies were rash and gastrointestinal disorders such as diarrhea, dry mouth, nausea and loose stools [116]. Ciardiaello et al., also reported good response in patients with metastatic breast cancer treated with gefitinib plus docetaxel, who had not previously received any kind of therapy for metastatic disease. The most common adverse effects in this study were neutropenia, diarrhea and nausea [117]. Interestingly, Osborne et al, found that the gefitinib plus tamoxifen combination was effective only in patients with newly diagnosed metastases or those who had recurring one year or more after stopping adjuvant therapy with tamoxifen but not in patients with recurrent disease during or after adjuvant treatment with aromatase inhibitors or those progressing after first-line aromatase inhibitor treatment for metastatic disease [118].

Additionally, Kalykaki et al., demonstrated that gefitinib was effective to reduce the number of EGFR positive and negative circulating tumor cells in patients with metastatic breast cancer [119].

The most common adverse effects of gefitinib as monotherapy in breast cancer patients are diarrhea, rush, dry skin, pruritus, dry mouth, nausea, vomiting and fatigue [108, 111, 116].

**Gefitinib resistance and toxicity**

The constitutive activation of signaling pathways downstream of EGFR contributes to gefitinib resistance [104, 120, 121]. Normanno et al., demonstrated that hiperactivation of the MAPK pathway is involved in both the intrinsic and acquired gefitinib resistance in breast cancer cells. Interestingly, blockade of the MAPK activity resulted in growth inhibition and induction of apoptosis. In addition, constitutive activation of p42-MAPK was related with gefitinib resistance [120, 121].

Other study related with intrinsic gefitinib resistance demonstrated that this TKI increased the expression of genes codifying for HER specific ligands and induced active import and nuclear accumulation of the HER ligand neuregulin, suggesting its possible transcriptional role [122]. Moreover, HER3 and consequently PI3K/AKT signaling evade inhibition by gefitinib in vitro and in vivo [123].

Regarding autophagy, gefitinib induced this cellular process in various gefitinib-sensitive and -insensitive breast cancer cell lines. Treatment with gefitinib in combination with agents that inhibit late-stage autophagy such as hydroxychloroquine or bafilomycin A1 increased efficacy of gefitinib in vitro and in vivo [107].

Alternatively, other studies suggest that the upregulation of the PI3K pathway caused by loss of PTEN activity is involved in gefitinib resistance in breast cancer [104, 124, 125]. In fact, in an EGFR overexpressing breast cancer cell line which lacks PTEN function, gefitinib abolished the phosphorylation of both EGFR and MAPK but not AKT [124]. Moreover, gefitinib treatment in cells that PTEN function was restored resulted in the inhibition of AKT, GSK3β and the translation repressor protein 4EBP1 phosphorylation [124], restoration of gefitinib sensitivity, arrest in the G1 phase [125], and relocalization of FOXO3a into the nucleus. This transcriptional factor is implicated in cell cycle arrest and apoptosis [126].

Other mechanisms implicated in gefitinib resistance in breast cancer involved the interplay between EGFR and different receptors. In this regard, hepatocyte growth factor receptor (MET), a tyrosine kinase receptor implicated in breast cancer progression, confers gefitinib resistance through increased EGFR phosphorylation induced by the activation of the kinase c-Src. In fact, an inhibitor of MET kinase activity decreased both EGFR phosphorylation and cell proliferation in the presence of gefitinib in HER2-positive and EGFR overexpressing breast cancer cells [127].
As with lapatinib, FAM83A dysregulation has been shown to promote gefitinib insensitivity in breast cancer cells [79].

The crosstalk between G-protein coupled receptors (GPCR) and EGFR [128]; K-RAS activation (a downstream effector of EGFR signaling) [129] and EGFR mutations [130] have all been involved with gefitinib resistance in lung cancer. In this regard, GPCRs-EGFR interaction has also been reported in breast cancer [131]. Moreover, K-RAS gene mutations were found in several adenocarcinomas [132-134]. Interestingly, EGFR mutations have not been reported neither in primary breast carcinomas nor in several breast cancer cell lines [135]. These issues have not yet been addressed in gefitinib resistant breast cancer cells and will have to be further analyzed.

**Neratinib**

Neratinib is another oral, but irreversible TKI, known as a pan-inhibitor because interacts with the catalytic domain of several EGFR family members (EGFR, HER2 and HER4) and blocks their downstream signaling pathways [136]. Neratinib covalently binds a specific and shared cysteine residue in the ATP-binding pocket of the receptors in the EGFR family [137]. In particular, neratinib binds to cysteine residues Cys-773 and Cys-805 in HER1 and HER2, respectively [138].

Neratinib derives from structural modifications of EKB-569, another potent and irreversible EGFR inhibitor [136, 139]. Neratinib has significant activity in naïve and previously exposed to trastuzumab patients [140], making it an alternative treatment for HER2-positive breast cancer. Currently, this TKI is in clinical trials and has been used to treat solid tumors and metastatic HER2 breast cancer [141-144].

**Mechanism of action of neratinib: preclinical evidence**

There are some reports that describe the mechanism of action of neratinib in breast cancer. A pioneering work from Rabindran et al., showed that neratinib inhibited proliferation and EGFR, HER2, HER4, AKT and MEK phosphorylation in HER2 overexpressing breast cancer cell lines [136, 145]. The regulation of downstream signal transduction by neratinib leads to arrest at the G1-S phase transition resulting in increased p27 levels and decreased phosphorylated retinoblastoma protein (pRb) and cyclin D1 expression. Interestingly, neratinib showed less antiproliferative activity in cell lines that express neither HER2 nor EGFR [136]. Moreover, HER2-positive breast cancer cell lines are more likely to respond to neratinib than EGFR-positive cells or HER2 non-amplified cell lines [145]. Another antineoplastic mechanism for neratinib in cancer cell lines is that it can reverse membrane-bound ATP transporters-mediated multidrug resistance [146]. The inhibition of multidrug resistance via ATP transporters by neratinib may be an alternative mechanism that could improve the response to chemotherapy agents used in HER2-positive breast cancer.

Similarly, neratinib enhanced the therapeutic response and counteracted trastuzumab resistance by decreased trastuzumab-induced HER4 nuclear translocation in HER2-positive breast cancer [145, 147]. A resume of neratinib mechanisms is found in Figure 1.

**Neratinib: clinical evidence and side effects**

Neratinib has proved to be a promising therapy for HER2 metastatic breast cancer in five recent phase I/II clinical studies listed in Table 2. Nevertheless, in a phase II trial, Martin et al. compared two treatments: neratinib versus lapatinib plus capecitabine with inconclusive results, since neither inferiority nor superiority of the monotherapy versus combination therapy could be demonstrated. However, they confirmed relevant single agent clinical activity and acceptable overall tolerability of neratinib [148]. These studies have warranted further studies of this TKI and its combination with other therapies. In this regard, several clinical trials phase I/II (ClinicalTrials.gov identifiers: NCT01608150, NCT01111825, NCT01570877, NCT00398567, NCT00741260, NCT009450-18, NCT01494662, NCT00146172) and two phase III (NCT00878370 and NCT01808573) in HER2-positive breast cancer population are in progress.

**Neratinib resistance and toxicity**

Seyhan et al., using a pool-based lentiviral genome-wide functional RNAi screen identified the following genes involved in neratinib resistance: oncogenesis (RAB33A, RAB6A and
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BCL2L14), transcription factors (FOXP4, TFEC, ZNF), cellular ion transporters (CLIC3, TRAPPC2P1, P2RX2), protein ubiquitination (UBL5), cell cycle (CCNF9) and genes known to interact with breast cancer-associated genes (CCNF, FOXP4, TFEC, several ZNF factors, GNA13, IGFBP1, PMEPA1, SOX5, RAB33A, RAB6A, FRX1, DDO, TFEC, OLFM2) [149]. This analysis helps to the identification of new biomarkers related to neratinib treatment that could eventually make more effective the use of combination targeted HER2 therapy.

In a similar manner as other TKIs, neratinib also may generate resistance. In this regard, NmU overexpression and low miR-630 levels are associated with innate or acquired-resistance to neratinib in HER2-positive breast cancer cells [77, 87].

In HER2-positive breast cancer cell lines, despite the initial inhibition of phosphorylation of all members of the EGFR family by neratinib, its continued exposure resulted in reactivation of HER3 and AKT signaling. This process is thought to be involved in the developing of resistance to neratinib. Interestingly, the combination of trastuzumab and neratinib blocked the reactivation of HER3 and AKT compared to neratinib alone [145].

Conclusion

Overexpression of EGFR family members and activation of their signaling pathways have been associated with the development of breast cancer and poor prognostic [150]. Interaction between these receptors is related with resistance to current therapy becoming a challenge for HER2-positive breast cancer treatment [100, 151]. Targeted therapy against cell signaling pathways of EGFR family members has been introduced in the clinic in recent years since it generates fewer side effects than conventional therapy [152]. In this regard, TKIs have been developed such as lapatinib, gefitinib and neratinib. These inhibitors have a similar mechanism of action; specifically, they compete for the ATP binding pocket of the EGFR family. These agents can inhibit the same signaling pathways such as PI3K/AKT, MAPK, PLCγ, and STAT [104, 153-155]. However, the differences in their responses, and their selectivity in HER2-positive breast cancer, could depend on their dissociation constant (kd) to EGFR family members, and their molecular interactions with these proteins. The biological consequences of the binding of TKIs with EGFR family members are poorly understood, and several researchers are currently examining the different TKIs interactions within the human kinase [156]. In addition, it has been described that TKIs can interact with different dimerization complexes with active or inactive forms of EGFRs [157-159]. Also, TKIs can modify the amount of EGFR family ligands [122].

However, if the three compounds have similar mechanisms of action, what is the difference between them that could explain the different responses in the clinic? A current work of Sánchez-Martín et al, in 2012 gives us an explanation about the chemical mechanism that led to increased efficacy of lapatinib and neratinib action in breast cancer versus gefitinib action. The reversible and irreversible inhibitors, lapatinib and neratinib; respectively, have in their structure an aromatic group that binds into the ATP pocket of the EGFR family while maintain it in a closed conformation, reducing the flexibility of the kinase receptor, which ultimately limits the dimerization with other receptors. Unlike lapatinib and neratinib, gefitinib lacks an aromatic group in its structure, which maintains EGFR family ATP pocket in an open conformation, favoring the dimerization with other kinase receptors [160].

Moreover, a recent work of O’Neill et al, in 2013 studied a panel of genes implicated in cell cycle progression, tumorigenesis and invasiveness, which were upregulated after treatment with neratinib in HER2 overexpressing breast cancer cells. This work demonstrated that the genic regulation induced by neratinib was similar to that seen with trastuzumab and the combination of lapatinib and capecitabine in HER2-positive breast cancer cells. In contrast, gefitinib treatment resulted in a completely different expression pattern [161, 162]. This is important because the expression of genes could be predictors of the response in some patients [161]. In summary, a different gene expression profile induced by each one of the TKIs could explain the difference in the treatment response.

The TKIs have shown sustainable features to block the signaling of the EGFR family members, inhibit cell proliferation and induce apop-
osis in HER2-positive breast cancer subtype. However, the search for novel therapeutic strategies comprising the use of these or other TKIs, in combination with different antineoplastic agents could further improve the prognostic in cancer patients. In order to enhance the clinical response to the TKIs therapy, we consider that tumor molecular analyses, which include the characterization of the EGFR family members, and the search for mutated or overexpressed proteins related with TKI resistance, must be performed.

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Disclosure of conflict of interest

None.

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