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DRUG EVALUATION

# Pharmacodynamics, pharmacokinetics and clinical efficacy of neratinib in HER2-positive breast cancer and breast cancer with HER2 mutations

Hampig Raphael Kourie*, Marie Chaix*, Andrea Gombos, Phillippe Aftimos and Ahmad Awada

Medical Oncology Clinic, Jules Bordet Institute, Free University of Brussels, Brussels, Belgium

**ABSTRACT**

**Introduction**: Despite the availability of several potent HER2-directed targeted agents, primary and acquired resistance continues to influence patient outcomes in HER2-positive breast cancer. Neratinib is an irreversible pan-HER tyrosine kinase inhibitor in late-phase clinical development.

**Areas covered**: This review article focuses on neratinib in the treatment of HER2-positive breast cancer – early and metastatic stage – and HER2-mutant breast cancer, with particular emphasis on the pharmacokinetics and pharmacodynamics of the drug.

**Expert opinion**: The phase III ExteNET trial shows that neratinib improves 2-year invasive disease-free survival after trastuzumab-based adjuvant therapy in early-stage HER2-positive breast cancer, and in particular HER2+/HR+ tumors. Survival data are awaited. The investigational role of neratinib in high-risk patients or conversely in de-escalation dual regimens with other anti-HER2 therapies and without chemotherapy are of interest. Phase II trials show that neratinib has efficacy, either as monotherapy or in combination with other chemotherapeutic or endocrine agents, in patients with HER2-positive metastatic breast cancer and in tumors harboring HER2 mutations. The role of neratinib in therapeutic algorithms of HER2-positive patients, as well as delaying CNS events, awaits the results of ongoing trials such as NALA. Diarrhea, the main toxicity of neratinib, can be effectively managed with early loperamide prophylaxis.

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## 1. Introduction

Overexpression of human epidermal growth factor receptor 2 (HER2) in breast cancer is associated with a more aggressive clinical phenotype and a poor prognosis [1–3]. Several novel HER2-directed targeted therapies have become available in the past 15 years and their use has improved overall survival in patients with early-stage [4,5] and metastatic disease [6,7] and transformed the treatment of HER2-positive breast cancer. Yet, some patients do not respond to these agents (primary resistance) and most metastatic patients eventually progress (acquired resistance) despite treatment [8–10]. Central nervous system (CNS) recurrences, a common site of metastasis in HER2-positive breast cancer [11], are also a particular and unique challenge in this tumor type. In an effort to improve outcomes and overcome resistance, several new HER2-targeted agents are currently in clinical development.

Neratinib (Puma Biotechnology Inc., Los Angeles, CA, USA) is a potent, orally administered irreversible pan-HER tyrosine kinase inhibitor with activity against HER1 (also referred to as epithelial growth factor receptor, EGFR), HER2, and HER4 [12]. The purpose of this article is to summarize the main pharmacodynamic and pharmacokinetic characteristics of neratinib, and its clinical development in the treatment of patients with HER2-positive breast cancers and those with HER2 mutations. A literature search of PubMed was done on 16 February 2016 using the following keywords: pharmacokinetics, pharmacodynamics, efficacy, neratinib, breast cancer. Furthermore, we identified relevant abstracts and presentations from major, and in particular, recent oncology meetings. Neratinib is also being investigated in other tumor types (i.e. HER mutation-positive or EGFR-amplified solid tumors, and quadruple wild-type [KRAS, NRAS, BRAF, PIK3CA] colorectal cancer), but these will not be discussed in the present review.

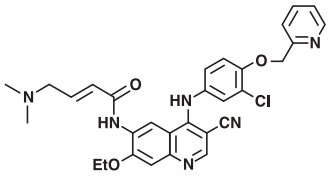
### 1.1. HER pathway in breast cancer

The family of HER (or ERBB) receptors is composed of four members, HER1 (EGFR, ERBB1), HER2 (ERBB2, HER2/*neu*), HER3 (ERBB3), and HER4 (ERBB4) [13], all of which have a function in controlling cell growth, proliferation, and survival. Structurally, each of them is located at the cell membrane and consists of two cysteine-rich extracellular ligand-binding domains, a single transmembrane domain and, with the exception of HER3, an intracellular tyrosine kinase domain [14] (Figure 1). Binding with ligands induces conformational changes in HER1, HER3, and HER4 [14] causing them to dimerize with either themselves (homodimerization) or other HER receptors (heterodimerization) and undergo phosphorylation of the tyrosine kinase [15]. This results in activation of several downstream intracellular signaling pathways that mediate cell growth and proliferation, of

**CONTACT** Ahmad Awada ahmad.awada@bordet.be Medical Oncology Clinic, Jules Bordet Institute, Free University of Brussels, Boulevard de Waterloo 121, B-1000 Brussels, Belgium

*These authors contributed equally to this work.

**Box 1.** Drug summary

| | |
|---|---|
| Drug name | Neratinib |
| Phase | III (HER2-positive breast cancer)<br>II (HER2-mutant breast cancer) |
| Indication | Early-stage and metastatic HER2-positive breast cancer |
| Pharmacology/ Mechanism of action | Potent irreversible inhibitor of HER1, 2, and 4 receptor tyrosine kinases |
| Route of administration | Oral |
| Chemical structure |  |
| Pivotal trials | ExteNET [48], NALA |

which the phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/Akt and mitogen-activated protein kinase (MAPK) pathways are the most important [16–18].

Several mechanisms can lead to aberrant hyperactivation of HER receptors, including overexpression and somatic mutations resulting in constitutive activation. HER2 is the most frequently altered gene of the HER family, and approximately 15–20% of all breast cancers have HER2 gene amplification or overexpression [13]. Although HER2 has no known ligand [19], it is constitutively in an open conformation ready for dimerization [20] and is the preferred partner for heterodimerization of the other HER receptors [21]. HER2 heterodimers (e.g. HER2/HER3) are more effective than HER2 homodimers in promoting carcinogenesis through activation of intracellular signaling pathways [22,23].

Sequencing studies have demonstrated that HER2 can also be affected by somatic mutations in HER2 non-amplified breast cancers [24,25]. These are thought to occur in approximately 2% of primary breast cancers [26]; although, the frequency of HER2 mutations in relapsed or metastatic breast cancers is currently unknown [25]. Many of these mutations are activating events leading to oncogenic transformation [25], and some mutations have been associated with a lack of response to HER2-directed treatments in the metastatic setting [27].

### 1.2. Current therapeutic approaches in HER2-positive breast cancer

Four HER2-directed targeted agents are currently approved for use in HER2-positive breast cancer: trastuzumab (Herceptin®, Genentech Inc., San Francisco, CA, USA), a humanized monoclonal antibody that binds to the extracellular domain of HER2; pertuzumab (Perjeta®, Genentech Inc., San Francisco, CA, USA), a humanized monoclonal antibody directed against domain II of the extracellular domain of HER2 that inhibits HER2-dimerization with other HER family members [28]; trastuzumab emtansine (T-DM1, Kadcyla®, Genentech Inc., San Francisco, CA, USA), an antibody–drug conjugate of trastuzumab and the anti-microtubule agent emtansine; and lapatinib (Tyverb®/Tykerb®, GlaxoSmithKline, Middlesex, UK), a reversible dual tyrosine kinase inhibitor that blocks HER1 and HER2 (Figure 1).

Current treatment guidelines recommend dual anti-HER2 blockade with pertuzumab plus trastuzumab plus a taxane as the preferred first-line regimen in metastatic HER2-positive breast cancer [29,30]. This is based on findings from the CLEOPATRA trial, which showed that the addition of pertuzumab to standard first-line therapy (i.e. trastuzumab plus docetaxel) significantly improved both progression-free survival (PFS) and overall survival in women with metastatic HER2-positive breast cancer [7,31,32]. Trastuzumab emtansine [33–36] and, if not available, trastuzumab plus chemotherapy [6,37,38] are recommended as second-line treatment options [29]. Trastuzumab plus chemotherapy [39], trastuzumab plus lapatinib [40,41], or lapatinib plus capecitabine [42,43] are reserved for use in trastuzumab-exposed disease [29].

In early-stage HER2-positive breast cancer, a 12-month course of trastuzumab with standard adjuvant chemotherapy

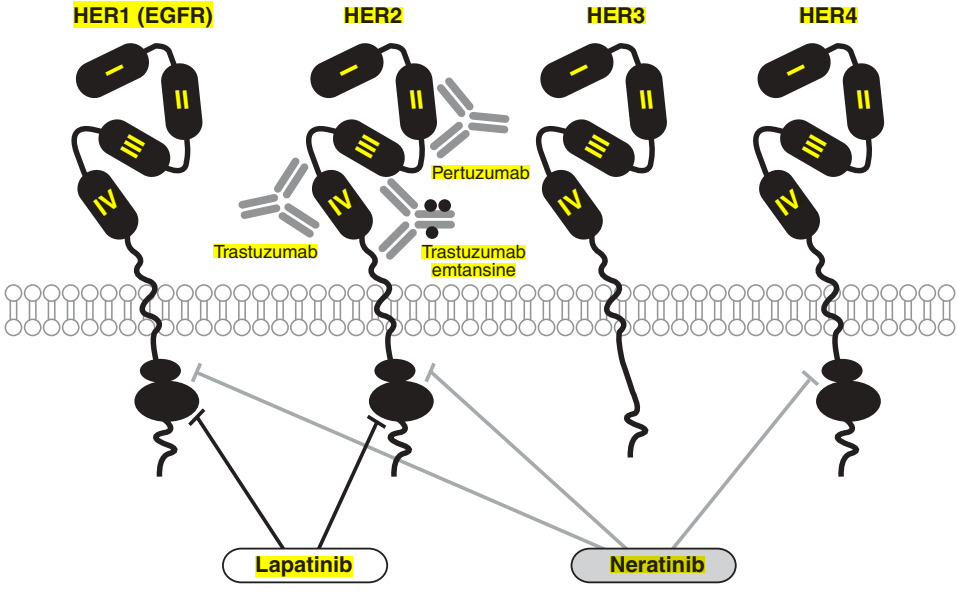



Figure 1. HER receptor family and site of action of trastuzumab, pertuzumab, trastuzumab emtansine, lapatinib and neratinib.

is recommended following surgery [29,44] based on the findings of landmark trials which showed that trastuzumab combined with chemotherapy approximately halves the recurrence risk compared with chemotherapy alone, translating into a 9% increase in 10-year overall survival [4,45,46]. In the neoadjuvant setting, dual therapy with pertuzumab and trastuzumab combined with standard chemotherapy improves pathological complete response rates (pCR) [47]. Pertuzumab and trastuzumab emtansine are currently being investigated in the adjuvant setting.

## 2. Introduction to neratinib

### 2.1. Chemistry

Neratinib (PB-272, HKI-272) is a 4-anilino-3-cyano quinoline derivative (IUPAC chemical name, (E)-*N*-[4-[3-chloro-4-(pyridin-2-ylmethoxy)anilino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide2-butenamide), as shown in Box 1. It is formulated as neratinib maleate (molecular weight, 673.11 g/mol).

### 2.2. Pharmacodynamics

Neratinib is a small-molecule irreversible tyrosine kinase inhibitor that was specifically designed to interact with HER2 kinase using a homology model for the catalytic domain of this enzyme [49]. Neratinib forms a covalent bond with a cysteine residue in the adenosine triphosphate (ATP)-binding pocket of HER receptor kinases (Cys773 in EGFR and Cys805 in HER2), a property that is thought to allow the agent to compete with high cellular concentrations of ATP and to provide extended inhibition of kinase activity [12]. As the cysteine residue required for binding is conserved in three of the HER receptors (i.e. EGFR, HER2, and HER4), neratinib is a pan-HER inhibitor of these receptors [50].

Neratinib is a potent and selective inhibitor of HER kinases *in vitro*. Neratinib has a high affinity for HER receptors and binds with a dissociation constant (Kd) of 1.1 nM for EGFR (HER1), 6.0 nM for HER2, and 2.4 nM for HER4 [51]. The mean concentration of neratinib required to inhibit receptor kinase activity by 50% [$IC_{50}$] was 92 nM for EGFR, 59 nM for HER2 [12], and 19 nM for HER4 in a cell-free autophosphorylation assay [52]. Neratinib had no significant activity against several other serine–threonine kinases or tyrosine kinases, suggesting that it is selective for HER kinases [12]. Neratinib consistently inhibited the growth of HER2- and EGFR-positive cell lines [12,53] and cell lines carrying activating HER2 mutations at nanomolar concentrations [25] (Table 1). The *in vitro* activity of neratinib was greater than that of lapatinib for all cell lines tested (Table 1).

Neratinib inhibits HER2 activation and downstream signal transduction in HER2-positive cell lines *in vitro*. In BT-474 cells, neratinib decreased receptor phosphorylation of EGFR ($IC_{50}$ 3 nM), HER2 ($IC_{50}$ 5 nM) [12], HER3 and HER4 ($IC_{50}$ values not reported) [53]. Neratinib inhibited phosphorylation of MAPK ($IC_{50}$ 2 nM) and Akt ($IC_{50}$ 2 nM) at concentrations similar to those observed in cell proliferation assays [12]. In addition, neratinib blocks cell cycle progression by repressing cyclin D1 expression, reducing phosphorylation of the retinoblastoma gene product, with a concurrent increase in p27 (an inhibitor of cell cycle progression). These effects result in $G_1$-S arrest and an increase in cells with sub-G1 DNA content, indicating apoptosis [12].

Neratinib significantly inhibits the growth of HER2-dependent (i.e. 3T3/*neu*, BT-474, SK-OV-3) and EGFR-amplified (i.e. A431) tumors in murine xenograft models at oral dosages ranging from 10 to 80 mg/kg/day compared with vehicle-treated controls [12]. Combination therapy with neratinib and trastuzumab had additive effects in a HER2-positive tumor xenograft model [53]. The *in vivo* activity of neratinib appeared to be dependent on HER2 or EGFR expression, as the drug had no significant antitumor activity in xenografts of cell lines that expressed low levels of these receptors (i.e. MCF-7

Table 1. *In vitro* inhibitory activity of neratinib and lapatinib against HER2- or EGFR-dependent cell lines or cell lines harboring HER2 mutations.

| | HER2-positive or EGFR-positive | | | | Activating HER2 mutations | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mean (±SE) $IC_{50}$ (nM) | | | | | Mean (±SE) $IC_{50}$ (nM) | |
| Cell line | Receptor expression | Neratinib | Neratinib | Lapatinib | Cell line | HER2 mutation | Neratinib | Lapatinib |
| 3T3 | Control | 700 ± 78 | – | – | MCF-10A | Wild-type | <2 | 400 ± 60 |
| 3T3/*neu* | HER2+ | 3 ± 0.14 | – | – | MCF-10A | G309A | <2 | 470 ± 50 |
| SK-Br-3 | HER2+ | 2 ± 0.18 | – | – | MCF-10A | V777L | <2 | 1070 ± 570 |
| BT-474 | HER2+ | 2 ± 0.06 | <5 | 16 ± 11 | MCF-10A | D769H | <2 | 980 ± 950 |
| A431 | EGFR+ | 81 ± 9 | – | – | MCF-10A | V842I | <2 | 650 ± 210 |
| MDA-MB-435 | Control | 960 ± 165 | – | – | MCF-10A | del. 755–759 | 2.1 ± 0.2 | 660 ± 90 |
| SW620 | Control | 690 ± 84 | – | – | MCF-10A | L7555 | 15 ± 6 | >10,000 |
| EFM-192A | HER2+ | – | <5 | 75 ± 2 | BT-474 | HER2+ | <2 | 31 ± 2 |
| HCC1569 | HER2+ | – | <5 | 3550 ± 715 | MCF7 | Control | >3000 | >10,000 |
| HCC1954 | HER2+ | – | <5 | 358 ± 64 | | | | |
| MDA-MB-361 | HER2+ | – | <5 | – | | | | |
| SK-BR-3 | HER2+ | – | <5 | 54 ± 8 | | | | |
| UACC-812 | HER2+ | – | <5 | 432 ± 116 | | | | |
| UACC-893 | HER2+ | – | <5 | 1211 ± 251 | | | | |
| SUM-225 | HER2+ | – | 10 ± 0 | 89 ± 52 | | | | |
| SUM-190 | HER2+ | – | 10 ± 0 | 38 ± 3 | | | | |
| UACC-732 | HER2+ | – | 650 ± 370 | 2629 ± 480 | | | | |
| Reference | | [12] | [53] | [54] | | [25] | | |

HER2: Human epidermal growth factor receptor 2; EGFR: epithelial growth factor receptor; $IC_{50}$: drug concentration that inhibits cell growth/proliferation by 50%; SE: standard error.

and MX-1) [12]. Repression of tumor growth *in vivo* was associated with inhibition of HER2 phosphorylation after a single oral 40 mg/kg dose of neratinib; phosphorylation was inhibited by 84% within 1 h, 97% at 6 h, and 43% over 24 h [12]. These data suggest that receptor phosphorylation is inhibited for 24 h, long after neratinib has been cleared, which is consistent with the irreversible inhibition of the compound [52].

Neratinib may be able to overcome some mechanisms thought to mediate resistance to other HER2-directed agents. Neratinib inhibited receptor phosphorylation and downstream signaling pathways in two cell lines with acquired trastuzumab resistance, and effectively inhibited cell growth in trastuzumab-resistant cell lines *in vitro* [53]. Neratinib also prevented trastuzumab-induced upregulation of HER4 *in vitro* and *in vivo*, a possible mechanism of trastuzumab resistance [55]. Neratinib demonstrated some activity (micromolar concentrations) in lapatinib-resistant BT-474 cells, the resistance mechanism was thought to be due to autocrine feedback involving heregulin-driven HER3-EGFR-PI3K–PDK1 signaling [56].

Neratinib may also reverse resistance to chemotherapeutic agents through interaction with ATP-binding cassette (ABC) transporter B1. Neratinib produced a concentration-dependent decrease in the $IC_{50}$s of several chemotherapeutic agents (doxorubicin, vincristine, and paclitaxel) in cells with ABCB1-mediated resistance *in vitro* [57]. Neratinib 20 mg/mg every 3 days also reversed ABCB1-mediated multidrug resistance to paclitaxel in a xenograft model [57].

### 2.3. Pharmacokinetics and metabolism

A summary of the pharmacokinetic data for neratinib at the recommended therapeutic dose of 240 mg/day [60] in healthy adult volunteers and patients with solid tumors is shown in Table 2. Pharmacokinetic data from the first-in-human phase I trial showed that neratinib 240 mg (given with food) is absorbed relatively slowly, with a mean time to peak concentration of 4 h in patients with solid tumors [60]. Exposure to neratinib (i.e. peak plasma concentrations, $C_{max}$, and area under the concentration–time curve to 24 h postdose, $AUC_{0-24\ h}$) increased in a dose-dependent manner over the 40–320-mg dose range, with no further increase in exposure evident when the dose was elevated further (320 or 400 mg). The mean accumulation ratio was 1.14 after repeated doses of neratinib 240 mg/day, indicating no major accumulation of the drug. After 21 days of treatment with neratinib 240 mg/day, mean $C_{max}$ was 74 ng/mL and mean $AUC_{0-24\ h}$ was 939 ng h/mL [60]. Steady-state plasma concentrations also remained constant after 2–6 months of continuous treatment with neratinib 240 mg/day in patients with cancer; mean trough concentrations ranged from 52 to 59 ng/mL ($n$ = 81) [61].

The mean apparent oral dose clearance (CL/*F*) of neratinib was 346 L/h and the volume of distribution was 5476 L [62], indicating extensive tissue distribution. The mean elimination half-life ($t_{1/2}$) after a 240-mg dose of neratinib with food was approximately 14 h [60] which supports a once-daily dosing regimen. Binding studies show that neratinib binds reversibly to human serum albumin [63]. The pharmacokinetics of neratinib in Japanese patients [64] was similar to those observed in a US population [60] (Table 2).

Neratinib exposure, when given in combination with vinorelbine, paclitaxel, or temsirolimus [65–67], was similar to that observed after neratinib monotherapy [60] (Table 2). The potential for interactions between neratinib and other chemotherapeutic or targeted agents (i.e. capecitabine, trastuzumab emtansine) is currently being evaluated in ongoing studies.

Preclinical data suggest that neratinib is metabolized mainly by cytochrome P450 (CYP) 3A4 [62]. Coadministration of neratinib with ketoconazole, a known strong CYP3A4 inhibitor, decreased neratinib CL/F by approximately fourfold (mean 87 vs. 346 L/h with neratinib alone) and increased neratinib exposure by more than threefold (mean $C_{max}$ 201

Table 2. Pharmacokinetics of neratinib 240 mg/day once daily as a single agent and in combination with other agents.

| | Healthy adults | | | Patients with solid tumors | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Neratinib 240 mg/day | | | Neratinib 240 mg/day | | Neratinib + vinorelbine[†] | Neratinib + paclitaxel[‡] | Neratinib + temsirolimus[§] |
| ***N*** | 25 | 22 | 59 | 3 | 10 | 46 | 22 | 4 |
| **Day 1** | | | | | | | | |
| $C_{max}$ (ng/mL) | 71.8 (34) | 55.3 (36) | 68 (40) | 75.9 (17) | 76.3 (41) | – | – | – |
| $t_{max}$ (h) | 6.0 (4.0–8.0) | 6.0 (4.0–8.0) | – | 4.0 (4, 5) | 5.9 (2.0–8.0) | – | – | – |
| $t_{1/2}$ (h) | 12.6 (45) | 11.7 (26) | – | 13.9 (8) | 14.3 (19) | – | – | – |
| $AUC_{0-24\ h}$ (ng h/mL) | 891 (28) | 903 (45) | 1236 (39) | 823 (35) | 1640 (48) | – | – | – |
| CL/*F* (L/h/kg) | – | 346 L/h (61) | – | – | 3.7 (97) | – | – | – |
| $V_z/F$ (L/kg) | – | 5476 L (59) | – | – | 65 (63) | – | – | – |
| **Steady state** | **Day 7** | | | **Day 21** | **Day 21** | **Day 8** | **Day 15** | **Day 22** |
| $C_{max}$ (ng/mL) | 73.1 (35) | – | – | 73.5 (37) | 81.5 (56) | 81.8 (57) | 68.6 (47) | 78.4 (53) |
| $t_{max}$ (h) | 6.0 (4.0–8.0) | – | – | – | 4.0 (2.0–7.9) | – | – | 3.6 (2.6–8.5) |
| $t_{1/2}$ (h) | 14.6 (38) | – | – | – | 22.7 (88) | – | – | – |
| $AUC_{0-24\ h}$ (ng h/mL) | 1060 (25) | – | – | 939 (34) | 1110 (59) | 1151 (49) | 1027 (49) | 1402 (56) |
| CL/*F* (L/h/kg) | – | – | – | – | 5.4 (74) | – | – | – |
| $V_z/F$ (L/kg) | – | – | – | – | 149 (74) | – | – | – |
| Reference | [58] | [62] | [59] | [60] | [64] | [65] | [66] | [67] |

Values are reported as means (% coefficient of variation), except for $t_{max}$ which is reported as the median (range). Except for Abbas et al. [62], neratinib was given with food in all studies.

$AUC_{0-24\ h}$: Area under the concentration–time curve from treatment initiation to 24-h postdose; CL/F: apparent oral dose clearance; $C_{max}$: maximum plasma concentration; $t_{1/2}$: elimination half-life; $t_{max}$: time to $C_{max}$; $V_z/F$: apparent volume of distribution.

[†]Neratinib 240 mg/day plus vinorelbine 25 mg/m$^2$ on days 1 and 8 of each 21-day cycle.

[‡]Neratinib 240 mg/day plus paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of each 28-day cycle.

[§]Neratinib 240 mg/day plus temsirolimus 15 mg once weekly of each 28-day cycle.

vs. 55 ng/mL; mean AUC 4660 vs. 903 ng h/mL) in healthy subjects [62]. The $t_{1/2}$ of neratinib also increased from 12 to 18 h when neratinib was given concomitantly with ketoconazole [62]. Dose adjustments may be needed if neratinib is given with potent CYP3A inhibitors.

## 2.4. HER2-positive breast cancer

### 2.4.1. Metastatic HER2-positive breast cancer

**2.4.1.1. Phase II studies.** Neratinib has been extensively investigated in a series of phase II trials in patients with HER2-positive metastatic breast cancer, both as a single agent and in combination with different chemotherapeutic and targeted agents (Table 3). Overall response rates with single-agent neratinib ranged from 29% to 40% in patients with previously treated HER2-positive metastatic breast cancer [60,61,68]. Single-agent neratinib 240 mg/day also showed substantial clinical efficacy in both trastuzumab-pretreated and trastuzumab-naïve patients [61]; the 16-week PFS rate was 59% in patients with prior trastuzumab treatment and 78% in trastuzumab-naïve patients, with objective response rates of 24% and 56%, respectively.

Considerably, higher overall response rates were observed when neratinib was combined with chemotherapeutic agents, notably paclitaxel (73%) [66] and capecitabine (63%) [70] (Table 3), findings that have prompted larger randomized trials of these combinations. The results from the first of these studies – NEfERT-T – have recently been reported [69] (Table 3). In this study, women with previously untreated HER2-positive metastatic breast cancer received either neratinib 240 mg/day ($n$ = 242) or trastuzumab 4 mg/kg then 2 mg/kg weekly ($n$ = 237) combined with paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 every 28 days. After a median follow-up of 23.0 months, median PFS, the primary study end point, was 12.9 months with neratinib–paclitaxel and 12.9 months with trastuzumab–paclitaxel (hazard ratio 1.02, 95% confidence intervals [CI] 0.81–1.27; $p$ = 0.894) [69]. There were also no statistically significant differences between treatment groups for three of five secondary efficacy end points (i.e. objective response rate, clinical benefit rate, and duration of response), suggesting that the two regimens have similar efficacy in the first-line treatment of patients with HER2-positive metastatic breast cancer.

A notable finding from the NEfERT-T study was that the incidence of symptomatic or progressive CNS recurrences was significantly lower in the neratinib–paclitaxel group (20 patients, 8.3%) compared with the trastuzumab–paclitaxel group (41 patients, 17.3%) [relative risk 0.48; 95% CI 0.29–0.79; $p$ = 0.002]. The time to CNS metastases was also significantly delayed with neratinib–paclitaxel compared with trastuzumab–paclitaxel (hazard ratio 0.45; 95% CI 0.26–0.78; $p$ = 0.004) [69]. Both CNS outcomes were prospectively defined secondary end points of the study. The efficacy of neratinib in patients with heavily pretreated progressive CNS metastases from HER2-positive breast cancer is also being investigated in a phase II study (Translational Breast Cancer Research Consortium 022). A CNS objective response rate of 8% was reported with single-agent neratinib 240 mg/day,

Table 3. Phase I and II clinical trials (single-arm or randomized) of neratinib as a single agent or in combination with other chemotherapeutic or targeted agents in patients with HER2-positive advanced or metastatic breast cancer.

| Study [name] | $N$ | Setting or study cohorts* | Treatment(s) | Overall response rate, $n$ (%) | Median (95% CI) progression-free survival (months) |
|---|---|---|---|---|---|
| **Single-agent neratinib** | | | | | |
| Wong et al. [60] | 25 | Prior trastuzumab | Neratinib 40–400 mg/day | 8/25 (32) | 3.6 (1.7–5.6) |
| Burstein et al. [61] | 136 | Prior trastuzumab | Neratinib 240 mg/day | 15/63 (24) | 5.1 (3.7–7.3) |
| | | No prior trastuzumab | | 36/64 (56) | 9.1 (7.1–12.7) |
| Martin et al. [68] | 233 | Prior trastuzumab (73%) | Neratinib 240 mg/day | 34/117 (29) | 4.5 (3.1–5.7) |
| | | | Lapatinib 1250 mg/day + capecitabine 2000 mg/m$^2$/day on days 1–14 of a 21-day cycle | 47/116 (41) | 6.8 (5.9–8.2) |
| **Combination therapy** | | | | | |
| Chow et al. [66] | 102 | ≤1 cytotoxic regimen | Neratinib 240 mg/day + paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle | 48/68 (71) | 14.5 (11.0–21.2) |
| | | 2–3 cytotoxic regimens | | 24/31 (77) | 12.0 (9.0–20.2) |
| Awada et al. [69] [NEfERT-T] | 479 | First-line | Neratinib 240 mg/day + paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle | 181/242 (75) | 12.9 (11.1–14.9) |
| | | | Trastuzumab 4 mg/kg then 2 mg/kg/week + paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle | 184/237 (78) | 12.9 (11.1–14.8) |
| Saura et al. [70] | 72 | Prior lapatinib | Neratinib 240 mg/day + capecitabine 1500 mg/m$^2$/day on days 1–14 of a 21-day cycle | 4/7 (57) | – |
| | | No prior lapatinib | | 39/61 (64) | 9.3 (7.0–15.2) |
| Awada et al. [65] | 79 | Prior lapatinib | Neratinib 240 mg/day + vinorelbine 25 mg/m$^2$ on days 1 and 8 of a 21-day cycle | 1/12 (8) | 5.2 (2.8–9.4) |
| | | No prior lapatinib | | 23/56 (41) | 11.0 (7.1–15.0) |
| Swaby et al. [71] | 37 | Prior trastuzumab | Neratinib 240 mg/day + trastuzumab 4 mg/kg then 2 mg/kg/week | –/33 (27) | 4.4 (3.4–7.4) |
| Jankowitz et al. [72] | 21 | Prior trastuzumab | Neratinib 120–240 mg/day + trastuzumab 4 mg/kg then 2 mg/kg/week + paclitaxel 80 mg/m$^2$ on days 1, 8, and 15 of a 28-day cycle | 8/21(38)† | 3.7 (Time to progression) |
| Gajria et al. [73] [10-005] | 82 | Prior HER2-directed therapies | Neratinib 240 mg/day + temsirolimus 8 mg/week | 11/37 (30) | 4.8 (3.0–11.0) |
| | | | Neratinib 240 mg/day + temsirolimus 8 or 15 mg/week | 11/37 (30) | – |
| Gandhi et al. [67] | 11‡ | – | Neratinib 120–240 mg/day + temsirolimus 15–75 mg/week | 2/11 (18) | – |

*Some studies grouped patients into cohorts according to previous treatments.
†Six patients not evaluable for response.
‡Ten patients with breast cancer had HER2-amplified tumors and had been previously treated with trastuzumab.

which did not meet the study threshold for success [74]; however, the study is continuing and will test neratinib in combination with capecitabine in this patient population.

Dysregulation of downstream signaling pathways, particularly the PI3K pathway, has been implicated as a mechanism of acquired resistance to HER2-targeted treatments. Combination therapy may provide clinical benefit in patients with trastuzumab–refractory HER2-positive metastatic breast cancer. Study 10-005 is investigating the combination of neratinib and the mTOR inhibitor temsirolimus in patients with HER2-positive metastatic breast cancer previously treated with 1–4 lines of HER2-directed therapy. In a preliminary analysis, the overall response rate was 30% (22 of 74 patients), which included patients with prior exposure to pertuzumab and trastuzumab emtansine [73]. Aberrant activation of the PI3K pathway, either through PIK3CA or PTEN mutation, did not preclude a response to the combination of neratinib plus temsirolimus [73].

*2.4.1.2. Ongoing trials.* Several phase II and III trials of neratinib in combination with chemotherapy or other targeted agents in patients with HER2-positive metastatic breast cancer are currently in progress (Table 4). NALA, an international randomized phase III registration trial, is comparing neratinib plus capecitabine with lapatinib plus capecitabine as third-line or later therapy in women with HER2-positive metastatic breast cancer. The co-primary end points of NALA are PFS and overall survival. This study will look also at CNS events, i.e. time to intervention for symptomatic metastatic CNS disease. Three phase II trials are further investigating the efficacy of neratinib plus trastuzumab emtansine as second-line therapy, neratinib plus the mTOR inhibitor temsirolimus in trastuzumab-refractory tumors, as well as neratinib plus capecitabine in patients with inoperable CNS metastases; preliminary data from two of these trials are discussed in Section 2.4.1.1.

### 2.4.2. Early-stage HER2-positive breast cancer

*2.4.2.1. Extended adjuvant therapy.* Neratinib has also been evaluated as extended adjuvant therapy following standard trastuzumab-based therapy in women with early-stage HER2-positive breast cancer in ExteNET, a large international, randomized, double-blind, placebo-controlled phase III trial. In this study, patients received a 1-year course of either oral neratinib 240 mg/day ($n$ = 1420) or matching placebo ($n$ = 1420) within 1–2 years of completing trastuzumab. Randomization was stratified according to hormone receptor status, nodal status, and trastuzumab adjuvant regimen. The primary study end point was invasive disease-free survival (iDFS), defined as the time from randomization to first occurrence of invasive ipsilateral tumor recurrence, invasive contralateral breast cancer, local/regional invasive recurrence, distant recurrence, or death from any cause.

In the primary analysis of ExteNET, conducted after 2 years of follow-up, there were 70 iDFS events in the neratinib group compared with 109 events in the placebo group in the intention-to-treat population (hazard ratio 0.67; 95% CI 0.50–0.91; $p$ = 0.0091) [48]. The 2-year iDFS rates were 93.9% for neratinib and 91.6% for placebo. Disease-free survival including ductal carcinoma *in situ*, a secondary study end point, was also significantly improved with neratinib compared with placebo (hazard ratio 0.63; 95% CI 0.46–0.84; $p$ = 0.0017). The other predefined secondary end points of time to distant recurrence, distant disease-free survival, and cumulative incidence of CNS recurrences, although trending positive, showed no statistically significant differences between groups. Overall survival data are not yet mature [48].

Table 4. Ongoing clinical trials with neratinib.

| Protocol (name) | Registration identifier | Phase | Target enrolment | Setting | Treatment | Primary end point(s) |
|---|---|---|---|---|---|---|
| **Metastatic HER2-positive breast cancer** | | | | | | |
| PUMA-NER 1301 (NALA) | NCT01808573 | III | 600 | Third-line or later | Neratinib + capecitabine vs. lapatinib + capecitabine | PFS (independently assessed) and OS |
| 10-005 | NCT01111825 | I/II | 99 | Trastuzumab-refractory | Neratinib + temsirolimus | MTD and ORR |
| TBCRC 022 | NCT01494662 | II | 105 | CNS metastases | Neratinib ± capecitabine | CNS ORR |
| NSABP FB-10 | NCT02236000 | I/II | 63 | Second-line | Neratinib + trastuzumab emtansine | ORR, safety, and tolerability |
| **Early-stage HER2-positive breast cancer** | | | | | | |
| 3144A2-3004-WW (ExteNET) | NCT00878709 | III | 2840 | Adjuvant (post-trastuzumab) | Neratinib vs. placebo | iDFS |
| PUMA-NER 6201 | NCT02400476 | II | 70 | Adjuvant (post-trastuzumab) | Neratinib + intensive loperamide prophylaxis | Incidence and severity of diarrhea |
| NSABP FB-7 | NCT01008150 | II | 141 | Neoadjuvant | Neratinib + trastuzumab + paclitaxel vs. neratinib + paclitaxel vs. trastuzumab + paclitaxel | pCR |
| **Metastatic HER2 mutation-positive breast cancer** | | | | | | |
| 201209135 | NCT01670877 | II | 70 | First- or later line | Neratinib ± fulvestrant | CBR |
| PUMA-NER 5201* (SUMMIT) | NCT01953926 | II | 292 | Incurable | Neratinib ± fulvestrant | ORR at 8 weeks |

CBR: Clinical benefit rate; CNS: central nervous system; MTD: maximum tolerated dose; iDFS: invasive disease-free survival; NSABP: National Surgical and Adjuvant Breast and Bowel Project; ORR: overall response rate; OS: overall survival; pCR: pathological complete response; PFS: progression-free survival.
*Multihistology signal-seeking trial including discrete cohorts of patients with HER2, HER3, or HER4 mutations or EGFR gene amplification.

An interesting finding from ExteNET was that neratinib appeared to offer benefit in hormone receptor-positive patients (hazard ratio for iDFS 0.51; 95% CI 0.33–0.77, $p = 0.0013$) but not in hormone receptor-negative patients (hazard ratio for iDFS 0.93; 95% CI, 0.60–1.43, $p = 0.74$) as part of a predefined subgroup analysis (test of interaction, $p = 0.054$) [48]. The effect of neratinib was also more pronounced in patients who were confirmed HER2-positive by central testing (hazard ratio for iDFS 0.51; 95% CI 0.33–0.77; $p = 0.0015$; $n = 1463$) [48].

Patient follow-up in the ExteNET trial is ongoing and further formal analyses are planned (i.e. 5-year iDFS and an event-driven analysis of overall survival). An exploratory 3-year analysis showed results consistent with the primary analysis (hazard ratio for iDFS 0.74; 95% CI 0.56–0.96; $p = 0.023$ for neratinib vs. placebo) [75]. The 3-year iDFS rates were 90.5% in the neratinib group and 88.6% in the placebo group [75].

*2.4.2.2. Neoadjuvant therapy.* Two phase II studies are investigating the use of neratinib in conjunction with standard neoadjuvant chemotherapy in early-stage breast cancer. In ISPY-2, a multicenter, randomized adaptive study [76], researchers found that neratinib met the predictive probability criterion for success in a phase III trial in the HER2-positive, hormone receptor-negative patient cohort (78% probability of success). In this cohort, the estimated pCR was 55% with neratinib plus standard chemotherapy versus 32% with trastuzumab plus standard chemotherapy [76]. The neratinib arm of the ISPY-2 study is now closed, although the study continues to investigate other agents.

Neratinib is also being investigated by the National Surgical Adjuvant Breast and Bowel Project (NSABP protocol FB-7) as part of an ongoing 3-arm study in the neoadjuvant setting in patients with HER2-positive breast cancer [77]. The first data from this study show that pCRs in the breast and lymph nodes were higher in patients who received neratinib plus trastuzumab plus paclitaxel (50.0%) compared with those treated with neratinib plus paclitaxel (33.3%) or trastuzumab plus paclitaxel (38.1%) [77]. In keeping with the findings from ISPY-2, pCR rates were consistently higher in patients with hormone receptor-negative tumors (73.7% vs. 46.2% vs. 57.1%) [77]. Patients' follow-up in this trial is continuing.

### 2.4.3. Breast cancers harboring somatic mutations of HER2

Two phase II trials of neratinib in HER2 mutation-positive metastatic breast cancer are currently in progress (Table 4) and several other trials are being planned. An interim analysis from one of these trials (SUMMIT) has recently been presented [78]. SUMMIT is an international open-label, phase II study evaluating neratinib in multiple cohorts of patients with solid tumors with activating HER2, HER3, or EGFR mutations or with EGFR gene amplification. In the HER2 mutation-positive breast cancer cohort ($n = 19$), an objective response at week 8 was documented in 6 (32%) patients, with clinical benefit in 8 (42%) patients. Median PFS was 4.0 (range, 2.0–4.0) months [78]. Tumors were HER2 non-amplified in all cases and patients had received a median of 4 (range, 0–11) previous treatments in the metastatic setting. Enrollment and patient follow-up is continuing.

## 2.5. Safety and tolerability

A summary of the most common treatment-emergent adverse events with neratinib 240 mg/day is presented in Table 5 [48]. Adverse events associated with neratinib are generally mild-to-moderate in severity, and grade 4 events are rare. The most common adverse events are gastrointestinal in nature (i.e. diarrhea, nausea, and vomiting), with diarrhea being the main dose-limiting toxicity of the drug. Neratinib does not appear to be associated with cardiac or pulmonary toxicities that are recognized class effects of EGFR-directed tyrosine kinase inhibitors; the incidence rates of cardiac (i.e. QT prolongation, decreases in left ventricular ejection fraction) and pulmonary adverse events (i.e. interstitial lung disease, pneumonitis, pulmonary fibrosis) with neratinib were similar to rates observed with placebo in the ExteNET trial [48].

Diarrhea, the predominant toxicity of neratinib, is generally mild-to-moderate in severity [48]. In the absence of antidiarrheal prophylaxis, most grade 3 diarrheal events occur in the first month of treatment, with a marked reduction in frequency thereafter [48]. Grade 3 diarrhea with neratinib tends to be acute with a median duration of 5 days per patient [48]. To better manage neratinib-associated diarrhea, loperamide prophylaxis given in conjunction with neratinib for the first cycle of treatment has been introduced in all new and ongoing trials of the drug [79]. Although the precise loperamide regimen varies from study–to-study, it typically involves administering loperamide 16 mg on day 1, with dose tapering to 12 mg/day and then 6–8 mg/day over the first cycle of treatment [79]. Preliminary data from trials including an intensive loperamide prophylaxis regimen show a marked reduction in the incidence of grade 3 diarrhea (ranging from 0 to 17%) [72,73,80] compared with the ExteNET trial (40%), in which diarrhea was managed only after the development of symptoms [48]. The effectiveness of loperamide prophylaxis in the prevention of neratinib-associated diarrhea is currently being investigated in a prospective multicenter phase II trial (Table 4).

Table 5. Frequent treatment-emergent adverse events with neratinib (≥10% of patients) [48] Reprinted from Lancet Oncology, 17(3), Chan, et al. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial, pp. 367–77, Copyright (2016), with permission from Elsevier.

| | Patients, *n* (%) | | | | | |
|---|---|---|---|---|---|---|
| | Neratinib ($N = 1408$) | | | Placebo ($N = 1408$) | | |
| Adverse event | Grade 1–2 | Grade 3 | Grade 4 | Grade 1–2 | Grade 3 | Grade 4 |
| Diarrhea | 781 (55) | 561 (40) | 1 (<1) | 476 (34) | 23 (2) | 0 |
| Nausea | 579 (41) | 26 (2) | 0 | 301 (21) | 2 (1) | 0 |
| Fatigue | 359 (25) | 23 (2) | 0 | 276 (20) | 6 (<1) | 0 |
| Vomiting | 322 (23) | 47 (3) | 0 | 107 (8) | 5 (<1) | 0 |
| Abdominal pain | 314 (22) | 24 (2) | 0 | 141 (10) | 3 (<1) | 0 |
| Headache | 269 (19) | 8 (1) | 0 | 269 (19) | 6 (<1) | 0 |
| Upper abdominal pain | 201 (14) | 11 (1) | 0 | 93 (7) | 3 (<1) | 0 |
| Rash | 205 (15) | 5 (<1) | 0 | 100 (7) | 0 | 0 |
| Decreased appetite | 166 (12) | 3 (<1) | 0 | 40 (3) | 0 | 0 |
| Muscle spasms | 157 (11) | 1 (<1) | 0 | 44 (3) | 1 (<1) | 0 |
| Dizziness | 143 (10) | 3 (<1) | 0 | 125 (9) | 3 (<1) | 0 |
| Arthralgia | 84 (6) | 2 (<1) | 0 | 158 (11) | 4 (<1) | 0 |

### 2.6. Regulatory affairs

Neratinib is currently preregistration, with regulatory submission to the US Food and Drug Administration and European Medicines Agency anticipated in 2016.

### 2.7. Conclusions

Neratinib is a small-molecule irreversible pan-HER tyrosine kinase inhibitor. It inhibits the growth of HER2-positive breast cancer cell lines and cell lines harboring HER2 mutations at nanomolar concentrations and is consistently more active than lapatinib *in vitro*. Neratinib has predictable dose-dependent pharmacokinetics, with no evidence of accumulation after repeat dosing. In the adjuvant setting, a 1-year course of neratinib after standard trastuzumab-based adjuvant therapy significantly improves iDFS compared with placebo in patients with early-stage HER2-positive breast cancer. In the first-line treatment of metastatic HER2-positive breast cancer, neratinib in combination with paclitaxel has similar efficacy to trastuzumab plus paclitaxel and may reduce CNS events. Preliminary data suggest that neratinib has promising activity in patients with HER2 non-amplified breast cancers carrying HER2 mutations. Neratinib is generally well tolerated and is not associated with the cardiac or pulmonary toxicities that can occur with other EGFR-directed tyrosine kinase inhibitors. Diarrhea, the predominant toxicity of neratinib, can be effectively managed with primary loperamide prophylaxis given for the first cycle of treatment. Active phase II and III trials continue to define which breast cancer settings and subtypes respond best to neratinib and which combination partners provide the best outcomes.

## 3. Expert opinion

Despite the availability of several effective HER2-directed targeted agents for the treatment of HER2-positive breast cancer, response rates in the first-line setting range from 50% to 80% [6,31,34,38] and from 20% to 50% in the second-line setting [33,39,42], highlighting the need for other treatment options. Many novel strategies are under clinical evaluation for the treatment of HER2-positive breast cancer, including mTOR inhibitors, anti-programmed death-1 inhibitors, HER2-directed antibody–drug conjugates, and HER-specific tyrosine kinase inhibitors. Two new-generation irreversible tyrosine kinase inhibitors have reached phase III trials – neratinib, a pan-HER inhibitor, and afatinib, a HER1 and HER4 inhibitor. While late-phase trials with afatinib in HER2-positive metastatic disease have been negative [81,82], available data on neratinib are positive and this agent now leads this avenue of research.

In the first phase III trial to provide data on neratinib – ExteNET – a 1-year course of neratinib after completion of standard trastuzumab-based adjuvant therapy significantly improved 2-year iDFS compared with placebo in women with HER2-positive early-stage breast cancer [48]. An exploratory analysis of ExteNET after 3 years was also consistent with the primary 2-year analysis [75]. As the only other trial that had attempted to improve on the standard of care (using trastuzumab for 2 years rather than 1 year) had a negative result [5], neratinib is the first agent that seems to improve outcomes beyond trastuzumab in this setting. Long-term patient follow-up in ExteNET, as well as survival data, will be important to determine whether or not the effects of neratinib are durable. In addition, further studies are needed to define which groups of patients and/or molecular subtypes will benefit most from neratinib. De-escalation regimens based on neratinib in combination with other HER2 therapies and without chemotherapy (or limited number of chemotherapy cycles) are of interest and should also be investigated.

How neratinib will fit into the metastatic setting is still being investigated in ongoing phase II and III trials, although there is already clear evidence to suggest that neratinib has efficacy in patients who have progressed on other HER2-directed agents. Early phase II trials indicated that single-agent neratinib has efficacy in trastuzumab-pretreated patients [60,61], and subsequent trials showed that this effect could be enhanced through combination with either chemotherapeutic agents (i.e. capecitabine, vinorelbine) [65,70] or temsirolimus [73]. NALA – an active phase III trial – is comparing neratinib in combination with capecitabine with standard third-line therapy, lapatinib plus capecitabine, in patients who have progressed after two or more HER2-directed regimens for metastatic disease. This trial will not only define how neratinib performs in this setting but will also determine how neratinib should be positioned in relation to lapatinib in the treatment of later-stage HER2-positive metastatic disease.

The potential of small-molecule tyrosine kinase inhibitors to cross the blood–brain barrier and possibly control CNS metastasis is a notable advance in the management HER2-positive breast cancer. Lapatinib has already shown very modest efficacy in CNS metastases [83,84] and several signals from recent trials with neratinib suggest that it too may be effective in patients with CNS metastases. Most notable are the data from the NEfERT-T trial which showed a significant reduction in the frequency of symptomatic or progressive CNS recurrences, as well as an improvement in the time to CNS events, with neratinib plus paclitaxel compared with trastuzumab plus paclitaxel [69]. NALA also includes a CNS-focused secondary end point (i.e. time to intervention for symptomatic CNS disease) and will provide some insight into the relative efficacy of neratinib and lapatinib in CNS metastases originating from HER2-positive breast cancer.

In each setting, the potential benefits of neratinib will need to be balanced against its tolerability profile. Diarrhea, the recognized dose-limiting toxicity of neratinib, is common, but any assessment of the occurrence of diarrhea with neratinib should be tempered by the fact that all early trials of this agent were without diarrheal prophylaxis or pre-emptive diarrhea management. As most diarrhea events with neratinib occur during the first weeks of therapy, primary loperamide prophylaxis given with the first cycle of treatment is now mandatory in all ongoing trials of the drug. Early data on the effectiveness of this strategy are encouraging and suggest that the occurrence of grade 3 diarrhea can be reduced to between 0% and 17% [79].

Activating HER2 mutations, which can occur in HER2 non-amplified breast cancer, may also be susceptible to neratinib.

*In vitro* data suggest that cells harboring these mutations are highly sensitive to neratinib [25], and early clinical data with neratinib in patients with breast cancers carrying HER2 mutations are promising [78]. Although these activating mutations are rare (approximately 2% of patients with breast cancer), this patient population could constitute a very interesting niche for the development of neratinib.

## Declaration of interest

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•• of considerable interest

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