Neratinib in HER-2-positive breast cancer: results to date and clinical usefulness

Arlene Chan

Ther Adv Med Oncol
2016, Vol. 8(5) 339–350
DOI: 10.1177/1758834016656494

***Abstract*:** The management of HER-2-positive breast cancer has improved significantly with the use of targeted agents to the HER-2 signaling pathway. Despite the improved survival achieved with the use of trastuzumab and chemotherapy in both the adjuvant and metastatic setting, patients may still recur or progress; whilst preclinical data demonstrate that these cancer cells remain addicted to the HER-2 oncogene. Neratinib, an oral small molecule tyrosine-kinase inhibitor has efficacy in the metastatic and adjuvant setting of patients who have previously received trastuzumab-based treatment. Diarrhea, being a class effect of tyrosine-kinase inhibitor, is the most common side effect seen following neratinib administration, but recent data suggests that a prophylactic loperamide regimen can reduce the incidence of grade 3 diarrhea. Phase I through to III clinical trials of neratinib will be reviewed, with discussion of the postulated mechanism underlying diarrheal events and its management.

Keywords: HER-2-positive breast cancer, neratinib, tyrosine kinase inhibitor

Introduction

Breast cancer cells that demonstrate overamplification of the HER-2 oncogene are known to have increased proliferation rates and cell survival. Furthermore, preclinical studies have shown these breast cancer cells demonstrate 'oncogene addiction' to this pathway, such that use of agents successfully inhibiting this pathway leads to improved clinical outcome [Weinstein and Joe, 2008]. Trastuzumab, a monoclonal antibody, was the first agent to be shown to be effective in this molecular subtype of breast cancer and its use in the clinical setting has significantly improved survival in patients with metastatic breast cancer [Olson *et al.* 2013], and importantly, has led to increased overall survival rates in patients with early breast cancer [Yin *et al.* 2011]. Despite the advances in the adjuvant setting, up to 26% of patients may still experience a breast cancer relapse as seen in the longer follow up of the pivotal adjuvant trastuzumab trials [Perez *et al.* 2014; Slamon *et al.* 2011; Goldhirsch *et al.* 2013] and thus more effective anti-HER2 agents are needed.

Neratinib

Neratinib was developed as a noncompetitive tyrosine kinase inhibitor (TKI) of adenosine triphosphate (ATP). It has been shown that covalent binding of neratinib to the cysteine residue-805 in the kinase domain of epidermal growth-factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER2) leads to kinase activity inhibition. It is structurally related to a potent inhibitor of EGFR, EKB-569, but demonstrates greater inhibition of both EGFR and HER-2 than EKB-569. The deactivation of the target tyrosine-kinase enzyme on binding with neratinib provides irreversible inhibition that persists even when the drug is no longer in the cellular environment [Wissner and Mansour, 2008].

The activity of neratinib, in inhibiting cellular proliferation, is greatest in cell lines which have high levels of HER-2 (BT474, SK-Br-3). However, even in cell lines with HER-2 overexpression there will be varying sensitivity to neratinib; which is postulated to relate to the presence of other HER receptors and their ligands.

Correspondence to:
Arlene Chan, MBBS, FRACP, MMed
Medical School Curtin University and Breast Cancer Research Centre, Hollywood Private Hospital, 909 Nedlands 6909, WA, Australia
arlenechan@me.com

Importantly, pathways downstream of the HER-2 receptor, namely the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3 kinase/Akt, are also inhibited by neratinib. Further, in BT474 cell lines, cyclin D1 downregulation and induction of p27 by neratinib leads to disruption of cell-cycle regulation and cellular arrest [Rabindran *et al.* 2004].

Phase I studies

Authors of an early phase I study of neratinib in 72 patients with solid tumors evaluated the safety and efficacy in a population of patients with metastatic cancer which expressed EGFR or HER-2 [Wong *et al.* 2009]. Advanced breast cancer patients with HER-2-positive disease as determined by immunohistochemistry 2+ or 3+ in ⩾10% of cells and with measurable disease by RECIST (response evaluation criteria in solid tumors) comprised 40% of the study population. All patients had previously received and progressed following standard systemic chemotherapy treatment (proportion of patients with HER-2 positive breast cancer having received prior trastuzumab was not stipulated). In the total population, the maximum tolerated dose was determined to be 320 mg, as four patients experienced grade 3 diarrhea at a daily dose of 400 mg. Pharmacokinetic analyses showed a mean t_{max} of between 3 and 6.5 hours following a single dose ranging from 40 mg to 400 mg, with a mean elimination half-life of 14 hours after a 240 mg dose, thus supporting a daily dosing of neratinib. Of 25 evaluable breast cancer patients, all of whom had previously received trastuzumab, anthracycline and taxane chemotherapy, a partial response or sustained stable disease (defined as ⩾24 weeks) was seen in eight (32%) and one (4%) patients, respectively. There was measurable reduction in the maximum tumor size from baseline in 13 (52%) breast cancer patients, where 11 of 13 patients had tumors which were HER-2 3+. All patients were evaluable for toxicity, with the most common ⩾ grade 3 events being diarrhea (32%), fatigue (4%) and vomiting (4%). Frequency of grade 3 or higher diarrhea was dose related with a 17% incidence at 180 mg and 83% incidence at 400 mg daily.

RNA interference (RNAi) studies have provided supportive evidence for the strong additive effect of combining neratinib with agents that result in microtubulin and DNA polymerase inhibition [Seyhan *et al.* 2012]. In SKBR-3 cells, the use of paclitaxel or vinblastine together with neratinib led to greater antiproliferative effect than with either chemotherapy or neratinib alone. This preclinical data led to the development of a phase I/II study of neratinib and paclitaxel (NP) [Chow *et al.* 2013]. In part one of this study, eight patients with advanced solid malignancy were treated in a dose-escalation study using a standard 3 + 3 cohort design to determine the maximal tolerated dose (MTD). The latter was determined to be neratinib 240 mg daily plus paclitaxel given intravenously on a weekly basis at a dose of 80 mg/m^2 for 3 out of 4 weeks. In part two of this study, 110 HER-2-positive [defined by fluorescent *in-situ* hybridization (ISH) or immunohistochemistry 3+] advanced breast cancer patients were treated with MTD, dose determined in part one, and included 71 patients who had only received one line of chemotherapy in the metastatic setting, no prior exposure to lapatinib and 25% had received prior trastuzumab (A), and 31 patients had received no more than three lines of chemotherapy with or without prior lapatinib and 39% had received prior trastuzumab (B). In those patients who had received prior trastuzumab, the time since receipt of trastuzumab was not stated. The overall objective response (ORR) in the phase II intent-to-treat population (n = 102) was 71% [95% confidence interval (CI), 60.7–79.2%; complete response (CR) 7%; partial response (PR) 64%]. By investigator assessment in 99 patients who had evaluable disease, the ORR was 73% (95% CI, 62.9–81.2%), comprising seven CRs, 65 PRs and an overall clinical benefit of 81% (CR, PR and stable disease ⩾24 weeks). An overall median duration of response of 73 weeks was achieved in the evaluable patients (77.1 weeks in group A and 56.3 weeks in group B). In a subset analysis by hormone-receptor status, an 84% ORR (95% CI, 71.2–92.2%) with a median progression-free survival (PFS) of 64 weeks (95% CI, 52.1–92.1) was noted in patients with hormone-receptor-positive breast cancer. This was in contrast to an ORR of 26% (95% CI, 43.2–73.7%) and median PFS of 47.7 weeks (95% CI, 29.9–91.9) in the hormone-receptor-negative cohort. The most common all-grade adverse events seen in the 110 patients were: diarrhea (92%), neuropathy (51%), alopecia (46%) and nausea (34%). Grade 3 or 4 diarrhea was seen in 29% with median cumulative duration of diarrhea being 35.5 days (range 1–737 days), with no patients ceasing treatment due to diarrhea. The study did not observe any unexpected toxicities that were not previously known to be associated with either neratinib or paclitaxel monotherapy.

Extending beyond a doublet combination of NP, the NSABP have reported results from a phase I study of neratinib, paclitaxel and trastuzumab. Key inclusion criteria included HER-2 positivity by either immunohistochemistry 3+ or gene amplification by ISH; prior treatment with trastuzumab and a taxane, evaluable disease, adequate organ function and left ventricular ejection fraction ⩾50%. Standard doses of paclitaxel and trastuzumab were administered (80 mg/m^2 weekly, for 3 out of 4 weeks and 4 mg/m^2 loading and weekly 2 mg/m^2, respectively) with three dose-escalation cohorts of neratinib at 120 mg, 160 mg and 240 mg daily. Patients receiving 240 mg daily were assessed as having excessive toxicity and thus a fourth cohort at 200 mg daily was added to the study. A total of 21 patients were recruited over a 55-week period, with 12 patients having also received prior lapatinib and three patients having received trastuzumab emtansine (T-DM1). The most frequent grade 3/4 adverse events seen were diarrhea (38%), electrolyte imbalance or fatigue (19%), dehydration (14%), nausea or neutropenia (10%) and stomatitis or thrombocytopenia (5%). Of 15 patients allocated to dosing cohorts 120 mg, 160 mg or 240 mg, eight patients (53.3%, 4 in the 120 mg cohort and 4 in the 160 mg and 240 mg cohorts) experienced grade 3 diarrhea either during cycles one or two ($n = 6$) or subsequent to cycle two ($n = 2$). With the implementation of a mandatory loperamide prophylaxis regimen (4 mg with the first dose of neratinib, followed by 2 mg every 4 hours during days 1–3, and 2 mg every 6–8 hours during days 4–21) in patients allocated to the 200 mg cohort, there were no observed episodes of grade 3 or 4 diarrhea and four of six patients experienced grade 1 or 2 diarrhea at some time during study treatment [Jankowitz *et al.* 2013]. A transient decline of ⩾10% in left-ventricular ejection fraction from baseline was seen in two patients, with only one patient experiencing asymptomatic decline in ejection fraction below 50%, which recovered without treatment modification. All patients were asymptomatic and had recovery to >50% at follow-up assessment. Efficacy in 15 evaluable patients demonstrated an ORR 38% (CR 2 and PR 6) and overall clinical benefit 52% (including CR, PR and stable disease ⩾24 weeks). It should be noted that one of the patients who experienced stable disease, had known cerebral metastases which did not progress over the 16 months of treatment. In the 13 patients who had received two anti-HER-2 agents, 6 patients achieved either a PR or stable disease. The response seen together with acceptable toxicity using a prophylactic loperamide regimen, led this 3-arm combination to be included in the NSABP-B7 neoadjuvant trial (see below).

Clinical synergism and good tolerability of trastuzumab and vinorelbine has previously been reported [Chan *et al.* 2006; Chan, 2007; Andersson *et al.* 2011]. Preclinical data suggest additive efficacy with microtubule inhibition and neratinib [Seyhan *et al.* 2012]. On the basis of this evidence, Awada and colleagues have reported results from a phase I/II, open-label study of intravenous vinorelbine 25 mg/m^2 on days 1 and 8, every 3 weeks, together with successive cohorts of neratinib at 160 mg or 240 mg daily. All patients had measurable metastatic, HER-2 positive amplified disease defined by a ratio of ⩾2.0 on fluorescent or chromogenic ISH, received at least one prior anticancer treatment including a minimum of 6 weeks of trastuzumab and had normal cardiac function. A total of 12 patients were included in the dose-finding phase I study with the MTD being established as neratinib 240 mg/day plus vinorelbine 25 mg/m^2 on days 1 and 8. The phase II component included 79 patients with HER-2-positive advanced breast cancer where 64 patients had received only one line of anti-HER-2 therapy (namely trastuzumab) and 15 (19%) had previously received lapatinib as well. There was no evidence of pharmacokinetic interaction. Safety data were reported for all patients ($n = 91$). The most frequent grade 3/4 adverse events were neutropenia (46%), where primary prophylaxis with granulocyte colony-stimulating factor was not used, and diarrhea (28%). No decline in left-ventricular ejection fraction was seen, whilst unspecified, clinically important alteration in electrocardiogram was reported in eight patients. Independent tumor-response assessment was possible in 56 patients. An ORR of 41% (95% CI, 28.1–55.0%) was seen with a clinical benefit rate (as defined above) of 70%, median duration of response being 52.7 weeks (range 11.6–96.1) and median PFS was 48 weeks (95% CI, 30.9–65.1). In those patients who had received prior lapatinib, the ORR, CBR and median PFS was 8%, 42% and 22.7 weeks (95% CI, 12–14), respectively. The authors concluded that the tolerability and efficacy seen warranted further evaluation of this combination [Awada *et al.* 2013].

Prior to the more recent development of anti-HER-2 agents such as pertuzumab and T-DM1 that have resulted in significant survival

improvements [Swain *et al.* 2013; Verma *et al.* 2012], lapatinib and capecitabine (LC) were shown to be effective in trastuzumab-treated metastatic HER-2-positive breast cancer patients [Geyer *et al.* 2006]. Thus the combination of neratinib and capecitabine was studied and recently reported in a phase I/II study [Saura *et al.* 2014]. A total of 33 patients with incurable, advanced solid malignancy were included in the dose-finding phase I cohort (including 16 breast cancer and 9 colorectal cancer patients). Following establishment of the MTD, a further 72 patients with HER-2-positive advanced breast cancer (defined as immunohistochemistry 3+ or HER-2 amplified by ISH alone or following an immunohistochemistry 2+ result) were treated at the MTD in the phase II component of the study. Patients included in the phase II component had to have received prior trastuzumab therapy for ⩾6 weeks in the metastatic or locally advanced setting, as well as received a taxane chemotherapy. Patients were not permitted to have received capecitabine previously, a prespecified group of patients were permitted prior lapatinib, and none were permitted to have received cumulative anthracycline dose beyond 400 mg/m^2 for doxorubicin or 800 mg/m^2 for epirubicin. Safety and tolerability were reported collectively for all patients recruited to phase I and phase II components of the study. Dose-limiting toxicities were seen with grade 4 diarrhea or grade 3 transaminase elevation in two patients receiving neratinib 240 mg/day plus capecitabine 2000 mg/m^2 per day for the standard 14 days every 3 weeks; and grade 3 asthenia and appetite or grade 3 diarrhea in two patients receiving neratinib 200 mg/day plus capecitabine 2000 mg/m^2 per day; the MTD was confirmed to be neratinib 240 mg/day plus capecitabine 1500 mg/m^2. The most commonly reported all-grade treatment-related adverse events reported in the entire study cohort (n = 105) were diarrhea (88%), palmar–plantar erythrodysesthesia (48%), nausea (37%), asthenia or fatigue (37%), vomiting (29%) and anorexia (26%). Grade 3/4 diarrhea occurred in 50–100% of patients within each of the dose-escalation cohorts; and was 26% in the phase II patients. Cardiovascular adverse events attributable to treatment included reduction in left-ventricular ejection fraction ⩾10% in two patients but no clinical cardiac failure was observed. Antitumor activity was only assessed in the phase II group with 68 of the 72 patients being assessable. There was an ORR of 64% (95% CI, 51–76%) and stable disease was seen in 8%. Patients who had received lapatinib previously experienced an ORR of 57% (95% CI, 18–90%), with one patient achieving a CR, and stable disease was seen in 14%. The median PFS was 40.3 weeks (95% CI, 30.3–66.0) and 35.9 weeks (95% CI, 18.9–60.1) in patients who had not received prior lapatinib or who had, respectively.

Several mechanisms have been put forward to explain trastuzumab resistance, with upregulation of the PI3K-AKT-mTOR (phosphatidylinositol 3-kinase, protein kinase B, mammalian target of rapamycin) pathway being considered an important pathway (Nahta and Esteva, 2006). The use of temsirolimus (an mTOR inhibitor) given with neratinib was evaluated in a phase 1/2 trial of 82 metastatic HER-2-positive breast cancer patients, all of whom had received prior trastuzumab (Gajria *et al.* 2014). The MTD of temsirolimus 8 mg weekly and neratinib 240 mg daily, was previously determined in 37 patients (group 1). Patients included in the dose-escalation cohort (group 2, n = 45) underwent biopsy of the metastatic lesion for biomarker assessment and commenced treatment with the MTD established for temsirolimus for the first 30 days, together with neratinib 240 mg daily, and where this was tolerated, the dose of temsirolimus was increased to 15 mg weekly. Patients had received a median of three lines of anticancer treatments previously (range 1–8). Results were available in 31 patients from group 1 and 27 patients from group 2 (6 and 10 patients, respectively, withdrew consent prior to tumor assessment and 8 patients in group 2 had not reached their first tumor assessment). PR was seen in 30% for both groups and stable disease in 43% and 35%, respectively. The duration of response was 3.0 and 7.4 months, with PFS of 4.8 months and not-yet reached in group 1 and 2, respectively. Activity was seen in patients who had prior treatment with pertuzumab and T-DM1 and early biomarker analysis demonstrated responses in patients whose tumors were shown to have PI3KC or PTEN mutations.

Metastatic breast cancer

Despite the clear improvements in progression-free and overall survival achieved with the introduction of the first anti-HER-2 agent, trastuzumab, it is known that despite an initial response to trastuzumab and taxane therapy in the first-line metastatic setting, the majority of patients will ultimately progress [Slamon *et al.* 2001; Marty *et al.* 2005]. The results of the Cleopatra trial established the superiority of docetaxel, trastuzumab and pertuzumab as first-line

treatment in 808 patients with centrally confirmed HER-2-positive metastatic breast cancer when compared with the control arm, who received trastuzumab and docetaxel. There was a significant overall survival benefit with a hazard ratio of 0.68 (95% CI, 0.56–0.84, $p < 0.001$) [Swain *et al.* 2015] and establishes this regimen as the recommended first-line therapy in the metastatic setting. However, 50% of patients receiving the dual anti-HER-2-based regimen will experience disease progression at 18.5 months. Further, only approximately 46% and 11% of patients had received prior adjuvant anthracycline-based chemotherapy and trastuzumab, respectively, which raises the question as to the efficacy of this regimen in patients who relapse in the post-2005 era following the publication of the pivotal adjuvant trastuzumab trials [Baselga *et al.* 2012].

In the setting of metastatic progression on trastuzumab and chemotherapy, the antibody–drug conjugate T-DM1 has been shown to provide significant survival benefits; both in the first-line metastatic setting, or following rapid relapse within 6 months of trastuzumab-based treatment [Verma *et al.* 2012] and after two anti-HER-2 agents [Krop *et al.* 2014]. The 991 patients entered into the EMILIA study had uniformly received trastuzumab and a taxane either within the period of the previous 6 months in the adjuvant setting or as first-line treatment in the metastatic setting. Despite the significant improvement in PFS in the EMILIA study, the median PFS for the T-DM1 cohort was 9.6 months, with an absolute median overall survival gain of 5.8 months and only 44% of patients achieving an objective response. In those patients who had received both trastuzumab and lapatinib in the metastatic setting, there was a significant increase in PFS with an absolute difference of 2.9 months but with >70% of patients having progression by 12 months on the study [Krop *et al.* 2014]. Although the development of the pertuzumab and T-DM1 has clearly added effective treatment options to patients with metastatic HER-2-positive disease, not all patients respond, and ultimately, disease progression occurs. Thus, identification of other effective anti-HER-2 agents is needed. In addition, these agents may not be readily available or available as reimbursed therapies in developing nations, which account for 53% of new cases of breast cancer annually [GLOBOCAN, 2012].

The first open-label multicenter study with neratinib monotherapy was reported in 2010. In the study, two cohorts of HER-2-positive (confirmed by ISH by an independent laboratory) metastatic breast cancer patients were eligible to receive neratinib 240 mg daily. Cohort A patients ($n = 66$) had received prior trastuzumab for at least 6 weeks, either as adjuvant therapy with disease recurrence occurring (six patients) or in the metastatic setting after one or two, or more than three trastuzumab-based regimens, which were 46 and 14 patients, respectively. Cohort B patients ($n = 70$) had not received prior trastuzumab therapy and were either chemotherapy naïve in the metastatic setting (34 patients) or had received up to three lines of cytotoxic regimens with an anthracycline (81%), taxane (53%) or both agents (65%) [Burstein *et al.* 2010]. A clinical benefit rate (defined as objective response or stable disease for ⩾24 weeks) of 33% and 69% was demonstrated in cohorts A and B, respectively. The primary objective of 16-week PFS by independent assessment was 59% (95% CI, 45–71%) and 78% (95% CI, 66–87%), respectively. Diarrhea of any grade was the most common adverse event, occurring in 93% of patients, with 21% of the events being of grade 3 or 4 severity. The median time to onset of diarrhea was 2–3 days, with median duration per episode of diarrhea being 5–7 days. Four patients (three with prior trastuzumab exposure) experienced a decline in left-ventricular ejection fraction below 50%. In total, two grade 3 cardiac events were reported (atrioventricular block and acute left-ventricular failure) and considered by the treating physician to be unrelated to neratinib. Pharmacokinetic studies in 81 patients, representative of both cohorts, demonstrated that daily dosing of neratinib achieved mean trough concentrations greater than the level required to inhibit autophosphorylation of HER-2 in BT474 HER-2 overexpressing cell line [Rabindran *et al.* 2004].

Due to poor recruitment, a planned phase III trial comparing neratinib monotherapy with LC was modified to a phase II design, with noninferiority in PFS being the primary endpoint. Eligible patients had HER-2 positivity confirmed by an immunohistochemistry score of 3+ or by ISH testing. Of the 233 patients recruited, 86% of patients had received two or more lines of prior anticancer treatment, with all patients having received trastuzumab previously, with 79% of the neratinib arm and 68% of the LC arm receiving trastuzumab in the metastatic setting. During this study, dose intensity was 100% for neratinib, 95% for lapatinib and 84% for capecitabine. On

an intent-to-treat analysis, patients receiving neratinib had a median PFS of 4.5 months (95% CI, 3.1–5.7) compared with 6.8 months for LC (95% CI, 5.9–8.2) resulting in an HR of 1.19 (95% CI, 0.89–1.6), which failed to meet the statistical significance for noninferiority that was set at an HR of 1.15. The most common grade 3 or 4 adverse effects in patients receiving neratinib were diarrhea (28% compared with 10% for LC) and palmar–plantar erythrodysesthesia in 10% of LC patients (nil for neratinib). The authors concluded that neratinib was not inferior to LC as the lower margin of the 95% confidence interval was below one, and that ongoing evaluation of neratinib for HER-2-positive breast cancer was warranted [Martin, 2013].

The results of a phase II randomized controlled trial evaluating NP against trastuzumab and paclitaxel (TP) was reported [Awada *et al.* 2016]. This multicenter study randomized 479 patients with HER-2-positive (by immunohistochemistry 3+ or ISH) metastatic or inoperable locally advanced breast cancer with measurable disease. Patients with stable central nervous (CNS) metastases were eligible, and response rate in the brain was a secondary endpoint, whilst PFS was the primary endpoint. The PFS of 12.9 months was identical in both arms (NP, 95% CI, 11.1–14.9, TP, 95% CI, 11.1–14.8), with an HR of 1.02 (95% CI, 0.81–1.27, $p = 0.894$). CNS disease was present in 6 NP and 12 TP patients at baseline. At a median follow up of 23 months, progressive CNS disease was seen in 20 (8%) and 41 (17%) of patients, respectively. Extracranial disease was controlled in 17 NP and 33 TP patients at the time of CNS progression. Relative risk of CNS progression was 0.48 (95% CI, 0.29–0.79, $p = 0.002$) in favour of patients receiving NP. The Kaplan–Meier estimate of time to symptomatic or progressive CNS disease was significantly longer in the NP arm, with HR of 0.45 (95% CI, 0.26–0.78, $p = 0.0036$). Any grade 3 or 4 adverse event was seen in 65% and 52% of patients, with diarrhea, 5.8% and 4.7%, being the most common event in NP and TP patients, respectively. Results from this study did not demonstrate that NP was superior to TP, but the significantly fewer CNS-progressive events deserves further evaluation.

Early breast cancer

As up to a quarter of patients who received adjuvant trastuzumab and chemotherapy may still experience a breast cancer recurrence, the ExteNET study was first implemented in 2009 in an attempt to assess for a reduction in risk of invasive breast cancer recurrences. Authors of this multicenter phase III trial aimed to evaluate the efficacy of neratinib given at its recommended 240 mg daily dose for 12 months compared with placebo in patients who had previously completed their adjuvant trastuzumab-based treatment, remained disease free, and were considered at ongoing risk of recurrence based on tumor stage at the time of diagnosis. The details of the protocol amendments during the history of the trial have been reported recently [Chan *et al.* 2016]. Patients were considered eligible if they had high-risk node-negative disease, node-positive or residual-invasive disease following neoadjuvant treatment (amended to only node-positive patients subsequently), completion of adjuvant trastuzumab up to 24 months earlier (amended to within 12 months subsequently), remained disease free and had a left-ventricular ejection fraction >50%, normal organ function and were able to comply with oral medication. The primary endpoint was invasive disease-free survival (DFS) (iDFS) with secondary endpoints including DFS for ductal carcinoma *in situ* and invasive disease, time to distant recurrence, distant DFS, cumulative incidence of CNS recurrences, overall survival and safety. The primary analysis demonstrated a significantly superior outcome for patients taking neratinib with a 2-year iDFS of 93.9% (95% CI, 92.4–95.2%) compared with placebo 91.6% (95% CI. 90.0–93.0%), translating to an HR 0.67 (95% CI, 0.50–0.91, $p = 0.0091$). Secondary endpoints of ductal carcinoma in situ (DCIS)-DFS were also superior for the neratinib arm with an HR of 0.63 (95% CI, 0.46–0.84, $p = 0.0017$). Efficacy in several predefined subgroups also demonstrated significant benefit with neratinib therapy, including iDFS in patients with hormone-receptor-positive disease with an HR of 0.51 (95% CI, 0.33–0.77, $p = 0.0013$) and those with centrally confirmed HER-2-positive disease ($n = 1705$) with an HR of 0.51 (95% CI, 0.33–0.77, $p = 0.0015$). In the absence of a protocol-specified anti-diarrheal prophylaxis program, 40% of patients in the neratinib arm reported grade 3 or 4 diarrhea (1 patient had a grade 4 event). The majority of patients experienced grade 3 diarrhea in the first 30 days of treatment, with a median time to onset of 8 days (range 4–33) and a median duration of 5 days (interquartile range (IQR), 2–9). All other grade 3 or 4 adverse events occurred in ⩽2% of patients receiving neratinib and there were no treatment-related deaths. An exploratory

analysis of iDFS at 3 years of follow up was reported at the 2015 San Antonio Breast Cancer Symposium [Chan *et al.* 2015]. The iDFS remained significantly different in favour of the neratinib arm with an HR of 0.74 (95% CI, 0.56–0.96). At the time of publication of the primary analysis, there was no significant difference in distant DFS and overall survival but long-term follow up continues.

In the neoadjuvant setting, the preliminary findings of the I-SPY2 trial have been reported [Carlson, 2014]. The significantly higher pathological CR rate in patients randomized to NP (39%) as compared with TP (23%), has led to the design of a phase III randomized-controlled trial to evaluate these two treatment arms.

Early results of the NSABP FB-7 study, which compared neoadjuvant TP with NP, with both trastuzumab and neratinib, and paclitaxel (TNP) in patients with locally advanced breast cancer was recently reported [Jacobs *et al.* 2015]. Patients with HER-2-positive disease as determined by immunohistochemistry 3+ score or ISH were randomized to weekly paclitaxel with trastuzumab, neratinib or both trastuzumab and neratinib for 4 months, with all patients then receiving four cycles of doxorubicin and cyclophosphamide prior to surgery. Pathological complete response (pCR) was 50% in the triplet arm in the overall population compared with 38.1% in trastuzumab and 33.3% in the neratinib arm. The highest pCR rates were seen in the hormone-receptor-negative patients (TNP 73.7%, TP 57.1% and NP 46.2%) as compared with the hormone-receptor-positive patients (TNP 30.4%, TP 29.6% and NP 27.6%). Grade 3 diarrhea occurred in 31% of TNP and NP patients, with none in TP. A subset of patients also received loperamide prophylaxis and in these 33 patients from the neratinib-containing arms, the incidence of grade 3 reduced to between 17% and 24%.

Toxicity

Diarrhea is the most common side effect seen in neratinib-treated patients with antagonism of the EGFR being considered the most likely mode of action underlying this toxicity. It has been shown that HER receptors are found in abundance on the basolateral membranes of epithelial cells of the intestine and are involved in the normal physiological maintenance of mucosal integrity [Van Sebille *et al.* 2015]. A number of hypotheses have been suggested to explain the underlying mechanism of diarrhea with administration of oral TKI. One mechanism relates to inhibition of EGFR, which then leads to direct mucosal atrophy as a result of suppressed enterocyte proliferation and re-epithelialization following injury. A second hypothesis relates to the role of HER receptors in intestinal ion transport, whereby HER-receptor dimerization leads to an inhibitory effect on chloride secretion and sodium absorption into the intestinal lumen. Inhibition of HER dimerization can then lead to an imbalance in translumen ion and water transport, which results in diarrhea [Hong *et al.* 2014; Van Sebille *et al.* 2015].

A pooled analysis of toxicity in 1468 lung cancer patients receiving EGFR-TKI in 21 trials conducted between 2006 and 2014 demonstrated an incidence of grade 3 or more diarrhea occurring in 0–33% of patients. Patients treated with afatinib had significantly higher rates of severe diarrhea when compared with those in trials evaluating gefitinib or erlotinib. The authors concluded that the higher affinity for the kinase domain of EGFR, and the sustained irreversible inhibitory effect of afatinib explained the greater severity of diarrhea with this agent [Takeda *et al.* 2015]. Lapatinib has been evaluated in the neoadjuvant, adjuvant and metastatic settings of patients with HER-2-positive breast cancer. The rates seen in the neoadjuvant setting without concomitant chemotherapy may provide the most accurate incidence of drug-related diarrhea, as patients are less likely to be on concomitant medications or experience myelosuppression with associated gastrointestinal infection. The use of neoadjuvant lapatinib was evaluated in the neoALTTO study, where 455 patients with HER-2-positive breast cancer, with tumors measuring more than 20 mm in size were randomized to lapatinib, trastuzumab or the combination. The incidence of all-grade diarrhea in the neoALLTO trial patients was 79%, with 23% of patients experiencing a grade 3 or 4 event [Azim *et al.* 2013]. One quarter of patients required dose reduction due to diarrhea and 5% discontinued treatment.

The frequency of diarrhea in published trials of neratinib with or without chemotherapy (21–40%, Table 1) has shown that the onset of severe diarrhea most commonly occurs in the first month of treatment and is generally of a short duration per episode (<7 days, Table 1). The incidence of diarrhea in clinical trials of neratinib in which no antidiarrheal prophylaxis was given compared

Table 1. Incidence of diarrhea in neratinib trials.

	n	Treatment	Diarrhea ⩾ 3	Median days to onset (range)	Led to discontinuation	Led to dose reduction	Median days of duration (range)
Wong *et al.* [2009]	72	Phase I dose-escalation study of neratinib 40–400 mg	32%	8.5 (1–22)	14%	86%	
Burstein *et al.* [2010]	136	Phase II neratinib monotherapy	21%	2–3		29%	5–7
Chow *et al.* [2013]	110	Phase I/II of neratinib 160 mg/240 mg + weekly paclitaxel	29%	3 (1–583)	24% (transient)	13%	36 (1–737)
Jankowitz *et al.* [2013]	21	Phase I paclitaxel, neratinib, trastuzumab	38%	<1–3			
Awada *et al.* [2013]	91	Phase I/II vinorelbine + neratinib	28%	7.3 (SD 14)	3%	15%	80 (SD 98)
Martin *et al.* [2013]	231	Phase II neratinib *versus* LC	28% (*versus* 10% LC)	3	2% (*versus* 4% LC)	12% (*versus* 1% LC)	3
Saura *et al.* [2014]	105	Phase I/II neratinib + capecitabine	23%	2* (1–164)	6%	10*	2* (1–418)
Awada *et al.* [2013]	479	Phase II neratinib/ paclitaxel *versus* trastuzumab/ paclitaxel	30.4%# (*versus* 3.8% TP)	16 (1–539)	3.80%		2 (1–15)
Chan *et al.* [2016]	2840	Phase III neratinib *versus* placebo	40%	8 (4–33)	16.80%	26.40%	5 (2–9)

*Results relate to patients in part two of the clinical trial.
#Three patients died of treatment-related adverse events.
LC, lapatinib and capecitabine; SD, standard deviation; TP, trastuzumab and paclitaxel.

with those with an intensive loperamide regimen has been published [Ustaris *et al.* 2015]. Loperamide exerts its effect by inhibiting basolateral colonic epithelial cells' potassium conductance, leading to diminished chloride secretion [Epple *et al.* 2001]. In an attempt to diminish the frequency of severe diarrhea, a prophylactic regimen using loperamide has been proposed as follows: a dose of 4 mg with the first dose of neratinib, followed by 2 mg every 4 hours for 3 days, then 2 mg given three to four times daily for the remainder of the first month of neratinib. Although patient numbers are small, trials using this loperamide prophylaxis have shown reduction in rates of grade 3 diarrhea to 8–12% in trials of neratinib monotherapy and 0–17% in trials where neratinib was combined with paclitaxel or temsirolimus [Ustaris *et al.* 2015]. Several articles have been published detailing the principles of managing diarrhea associated with the administration of TKI. These involve both a patient-education approach together with prophylactic loperamide, as well as prompt medical review and hospitalization for parenteral fluid and electrolyte replacement, with antibacterial medications where appropriate [Hirsh *et al.* 2014; Andreyev *et al.* 2014].

Adverse events of grade 1–4 seen in >20% of patients treated with neratinib included nausea (30%), vomiting (31%), fatigue (24%) and headache (20%) in the phase II trial in metastatic breast cancer patients [Burstein *et al.* 2010]. However, a more accurate assessment of drug-related side effects is seen in the ExtNET study where patients with HER-2-positive, predominantly node-positive early breast cancer who remained disease free, were randomized to receive neratinib as monotherapy or placebo. Cardiac toxicity is clearly an important side effect to assess for, as the higher incidence of cardiac dysfunction following treatment with anti-HER-2 monoclonal antibody treatment is well established [Guglin *et al.* 2009; Telli *et al.* 2007]. Albeit with short follow up of 2 years at the time of primary analysis, the ExteNET study demonstrated low rates of

Table 2. Ongoing clinical trials of neratinib for breast cancer.

ClinicalTrials.gov identifier	Title	Phase	Inclusion	Treatment	Outcome
NCT01808573	A study of neratinib plus capecitabine *versus* lapatinib plus capecitabine in patients with HER2+ metastatic breast cancer who have received two or more prior HER2 directed regimens in the metastatic setting (NALA)	III	Two or more anti-HER-2	Neratinib 240 mg + capecitabine 1500 mg or 750 mg *versus* lapatinib 1250 mg + 2000 mg or 1000 mg	Independent assessment of PFS
NCT02236000	A dose-escalation study evaluating the combination of trastuzumab emtansine (T-DM1) with neratinib in women with metastatic HER2-positive breast cancer (NSABP-FB-10)	I and II	Only one line of anti-HER-2 therapy (trastuzumab or trastuzumab and pertuzumab	Part 1: MTD of neratinib, Part 2: neratinib + T-DM1	Safety and tolerability
NCT01494662	Neratinib, capecitabine, and trastuzumab in treating patients with HER2-positive breast cancer that has spread to the brain	II	No prior capecitabine	Cohort 1: neratinib 240 mg, Cohort 2: neratinib day 7–21 then craniotomy, Cohort 3: add trastuzumab if extra-cranial progression	ORR in CNS
NCT02400476	A study looking the incidence and severity of diarrhea in patients with early-stage HER2+ breast cancer treated with neratinib and loperamide	II	Inclusion/ exclusion criteria as for ExteNET study	Neratinib 240 mg daily for 12 months	Incidence and severity of diarrhea
NCT01670877	Neratinib with or without fulvestrant in metastatic HER2-negative but HER2 mutant breast cancer	II		Neratinib 240 mg daily ± fulvestrant 500 mg monthly (and C1D15)	Overall clinical activity
NCT01953926	An open-label, phase II study of neratinib in patients with solid tumors with somatic human epidermal growth factor receptor (EGFR, HER2, HER3) mutations or EGFR gene amplification	II	Histologically confirmed cancers for which no curative therapy exists, documented HER2 mutation.	Neratinib 240 mg daily	Overall response rate at 8 weeks

PFS, progression-free survival; ORR, objective response rate; CNS, central nervous system; EGFR, epidermal growth-factor receptor; MTD, maximal tolerated dose.

cardiac dysfunction including symptomatic decrease in left-ventricular ejection fraction (1%) and QT prolongation (3%). With respect to other grade 3 or 4 nondiarrheal side effects occurring in 1–5% of patients, these included vomiting, nausea, abdominal pain and fatigue. All other grade 3 or 4 nondiarrheal side effects occurred in <1% of patients [Chan *et al.* 2016].

Future direction

The prognosis of patients diagnosed with HER-2-positive breast cancer has improved substantially with the use of anti-HER-2-targeted agents dating back to the first clinical trial results in the late 1990s [Cobleigh *et al.* 1999]. Preclinical and clinical evidence confirm the important role for ongoing HER-2 inhibition as being needed to achieve disease control despite recurrence or progression following trastuzumab-based therapy. Neratinib has been shown in the metastatic, neoadjuvant and adjuvant setting to be an effective treatment, both as a single agent or in combination with cytotoxic agents. The activity of neratinib was recently reported from the SUMMIT study in which activity in HER-2 nonamplified tumors,

which harbor somatic ERBB2 mutations, was seen, and suggests that it may have a role beyond HER-2 amplified breast cancer [Hyman *et al.* 2015]. At present, there are early but statistically significant benefits with its use as delayed adjuvant therapy, whilst preventative measures continue to be evaluated to reduce the rate of the commonest adverse effect, diarrhea. The improvement in hormone-receptor-positive early breast cancer in the ExteNET study is of particular interest as it contrasts with the lack of differential benefit in this patient group with other anti-HER2 agents and the mechanism underlying this effect is awaited. Further, the benefit seen in CNS progression in the NEfERT-T study (in which 479 HER-2-positive metastatic breast cancer patients were randomized in the first-line metastatic setting to NP or the control arm, TP) may suggest a population of patients where this drug has greater utility. The ongoing evaluation of this agent in several clinical trials (Table 2) will provide guidance as to its role amongst other anti-HER-2 agents in patients with breast cancer.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest statement

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Honorarium from Pfizer, Amgen, Eisai and Puma Biotechnology for educational meetings.

References

Andersson, M., Lidbrink, E. and Bjerre, K. (2011) Phase III randomized study comparing docetaxel plus trastuzumab with vinorelbine plus trastuzumab as first-line therapy of metastatic or locally advanced human epidermal growth factor receptor 2–positive breast cancer: The HERNATA Study. *J Clin Oncol* 29: 264–271.

Andreyev, J., Ross, P., Donnellan, C., Lennan, E., Leonard, P. *et al.* (2014) Guidance on the management of diarrhoea during cancer chemotherapy. *Lancet Oncol* 15: e447–e460.

Awada, A., Colomer, R., Bondarenko, I., Inoue, K., Badwe, R., Demetriou, G. *et al.* (2016) Neratinib plus paclitaxel *versus* trastuzumab plus paclitaxel in previously untreated metastatic HER2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA Oncol* 14 April 2016. [Epub ahead of print]

Awada, A., Dirix, L., Manso Sanchez, L., Xu, B., Luu, T. and Diéras, V. (2013) Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. *Ann Oncol* 24: 109–116.

Azim, H., Agbor-Tarh, D., Bradbury, I., Dinh, P., Baselga, J., Di Cosimo, S. *et al.* (2013) Pattern of rash, diarrhea, and hepatic toxicities secondary to lapatinib and their association with age and response to neoadjuvant therapy: Analysis from the NeoALTTO Trial. *J Clin Oncol* 31: 4504–4511.

Baselga, J., Cortés, J., Kim, S., Im, S., Hegg, R., Im, Y. *et al.* (2012) Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 366: 109–119.

Burstein, H., Sun, Y., Dirix, L., Jiang, Z., Paridaens, R., Tan, A. *et al.* (2010) Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J Clin Oncol* 28: 1301–1307.

Carlson, R. (2014) I-SPY 2 Trial: Neoadjuvant neratinib improves pathologic complete response in HR-/HER2+ breast cancer. *Oncol Times* 36: 25–26.

Chan, A. (2007) A review of the use of trastuzumab (Herceptin) plus vinorelbine in metastatic breast cancer. *Ann Oncol* 18: 1152–1158.

Chan, A., Delaloge, S., Holmes, F., Moy, B., Iwata, H., Harvey, V. *et al.* (2015) Neratinib after adjuvant chemotherapy and trastuzumab in HER2-positive early breast cancer: primary analysis at 2 years of a phase III, randomized, placebo-controlled trial (ExteNET). *J Clin Oncol* 33 (abstract 508).

Chan, A., Delaloge, S., Holmes, F., Moy, B., Iwata, H., Harvey, V. *et al.* (2016) Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase III trial. *Lancet Oncol* 17: 367–377.

Chan, A., Martin, M., Untch, M., Gil, M. and Guillem-Porta, V. (2006) Vinorelbine plus trastuzumab combination as first-line therapy for HER 2-positive metastatic breast cancer patients: an international phase II trial. *Br J Cancer* 95: 788–793.

Chow, L., Xu, B., Gupta, S., Freyman, A., Zhao, Y. and Abbas, R. (2013) Combination neratinib (HKI-272) and paclitaxel therapy in patients with HER2-positive metastatic breast cancer. *Br J Cancer* 108: 1985–1993.

Cobleigh, M., Vogel, C., Tripathy, D., Robert, N., Scholl, S., Fehrenbacher, L. *et al.* (1999) Multinational study of the efficacy and safety of humanized ant-HER2 monoclonal antibody in women

who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 17: 2639–2648.

Epple, H., Fromm, M., Riecken, E. and Schulzke, J. (2001) Antisecretory effect of loperamide in colon epithelial cells by inhibition of basolateral K+ conductance. *J Scand J Gastroenterol* 36: 731–737.

Gajria, D., Modi, S., Saura, C., Sakr, R., Solano, K., Won, H. *et al.* (2014) *A Phase I/II Study of Neratinib Plus Temsirolimus in HER2+ Metastatic Breast Cancer Reveals Ongoing HER2 Pathway Dependence in Many Patients Despite Several Lines of HER2 Targeted Therapy. Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium*, 9–13 December 2014, San Antonio, TX.

Geyer, C., Forster, D., Lindquist, D., Chan, S., Romieu, G., Pienkowski, T. *et al.* (2006) Lapatinib plus capecitabine for Her2-positive advanced breast cancer. *N Engl J Med* 355: 2733–2743.

GLOBOCAN. (2012). Available at: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx (accessed 19 December 2013).

Goldhirsch, A., Gelber, R., Piccart-Gebhart, M., De Azambuja, E., Procter, M., Suter, T. *et al.* (2013) 2 years *versus* 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. *Lancet* 382: 1021–1028.

Guglin, M., Hartlage, G., Reynolds, C., Chen, R. and Patel, V. (2009) Trastuzumab-induced cardiomyopathy: not as benign as it looks? A retrospective study. *J Card Fail* 15: 651–657.

Hirsh, V., Blais, N., Burkes, R., Verma, S. and Croitoru, K. (2014) Management of diarrhea induced by epidermal growth factor receptor tyrosine kinase inhibitors. *Curr Oncol* 21: 329–336.

Hong, S., Gu, Y., Gao, Z., Guo, L., Guo, W., Wu, X. *et al.* (2014) Pigment epithelium-derived factor (PEDF) inhibits breast cancer metastasis by down-regulating fibronectin. *Breast Cancer Res Treat* 148: 61–72.

Hyman, D., Piha-Paul, S., Rodón, J., Saura, C., Puzanov, I., Shapiro, G. *et al.* (2015) Neratinib for ERBB2 Mutant, HER2 Nonamplified, Metastatic Breast Cancer: Preliminary Analysis from a Multicenter, Open-Label, Multi-Histology Phase II Basket Trial. *Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium*, 8–12 December 2015, San Antonio, TX.

Jacobs, S., Robidoux, A., Garcia, J., Abraham, J., La Verde, N., Orcutt, J. *et al.* (2015) NSABP FB-7: A Phase II Randomized Trial Evaluating Neoadjuvant Therapy with Weekly Paclitaxel (P) Plus Neratinib (N) or Trastuzumab (T) or Neratinib and Trastuzumab (N+T) Followed by Doxorubicin and Cyclophosphamide (AC) with Postoperative T in Women with Locally Advanced HER2-Positive Breast Cancer. *Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium*, 8–12 December 2015, San Antonio, TX.

Jankowitz, R., Abraham, J., Tan, A., Limentani, S., Tierno, M. and Adamson, L. (2013) Safety and efficacy of neratinib in combination with weekly paclitaxel and trastuzumab in women with metastatic HER2-positive breast cancer: An NSABP Foundation Research Program phase I study. *Cancer Chemother Pharmacol* 6: 1205–1212.

Krop, I., Kim, S., González-Martín, A., Lorusso, P., Ferrero, J., Smitt, M. *et al.* (2014) Trastuzumab emtansine *versus* treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): A randomised, open-label, phase III trial. *Lancet Oncol* 15: 689–699.

Martin, M., Bonneterre, J., Geyer, C., Jr, Ito, Y., Ro, J., Lang, I. *et al.* (2013) A phase two randomised trial of neratinib monotherapy versus lapatinib plus capecitabine combination therapy in patients with HER2+ advanced breast cancer. *Eur J Cancer* 49: 3763–3772.

Marty, M., Cognetti, F., Maraninchi, D., Snyder, R., Mauriac, L., Tubiana-Hulin, M. *et al.* (2005) Efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: results of a randomized phase II trial by the M77001 Study Group. *J Clin Oncol* 23: 4265–4274.

Nahta, R. and Esteva, F. (2006) HER2 therapy: molecular mechanisms of trastuzumab resistance. *Breast Can Res* 8: 215.

Olson, E., Najita, J., Sohl, J., Arnaout, A., Burstein, H., Winer, E. *et al.* (2013) Clinical outcomes and treatment practice patterns of patients with HER2-positive metastatic breast cancer in the post-trastuzumab era. *Breast* 22: 525–531.

Perez, E., Romond, E., Suman, V., Jeong, J., Sledge, G. and Geyer, C. (2014) Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2–positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. *J Clin Oncol* 32: 3744–3752.

Rabindran, S., Discafani, C., Rosfjord, E., Baxter, M., Floyd, M. and Golas, J. (2004) Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res* 64: 3958–3965.

Saura, C., Garcia-Saenz, J., Xu, B., Harb, W., Moroose, R. and Pluard, T. (2014) Safety and efficacy of neratinib in combination with capecitabine in patients with metastatic human epidermal growth factor receptor 2–positive breast cancer. *J Clin Oncol* 32: 3626–3633.

Seyhan, A., Varadarajan, U., Choe, S., Liu, W. and Ryan, T. (2012) A genome-wide RNAi screen identifies novel targets of neratinib resistance leading to identification of potential drug resistant genetic markers. *Mod Biosyst* 8: 1553–1570.

Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M. and Press, M. (2011) Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med* 365: 1273–1283.

Slamon, D., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A. *et al.* (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783–792.

Swain, S., Baselga, J., Kim, S., Ro, J., Semiglazov, V., Campone, M. *et al.* (2015) Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med* 372: 724–734.

Swain, S., Kim, S., Cortes, J., Ro, J., Semiglazov, V., Campone, M. *et al.* (2013) Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase III study. *Lancet Oncol* 14: 461–471.

Takeda, M., Okamoto, I. and Nakagawa, K. (2015) Pooled safety analysis of EGFR-TKI treatment for EGFR mutation-positive nonsmall cell lung cancer. *Lung Cancer* 88: 74–79.

Telli, M., Hunt, S., Carlson, R., Guardino, A. *et al.* (2007) Trastuzumab-related cardiotoxicity: Calling into question the concept of reversibility. *J Clin Oncol* 25: 3525–3533.

Ustaris, F., Saura, C., Di Palma, J., Bryce, R., Moran, S., Neuman, L. *et al.* (2015) Effective Management and prevention of neratinib-induced diarrhea. *Am J Hematol Oncol* 11: 13–22.

Van Sebille, Y., Gibson, R., Wardill, H. and Bowen, J. (2015) ErbB small molecule tyrosine kinase inhibitor (TKI) induced diarrhoea: Chloride secretion as a mechanistic hypothesis. *Cancer Treat Rev* 41: 646–672.

Verma, S., Miles, D. and Gianni, L. (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 367: 1783–1791.

Weinstein, I. and Joe, A. (2008) Oncogene addiction. *Cancer Res* 68: 3077–3080.

Wissner, A. and Mansour, T. (2008) The development of HKI-272 and related compounds for the treatment of cancer. *Arch Pharm (Weinheim)* 341: 465–477.

Wong, K., Fracasso, P., Bukowski, R., Lynch, T., Munster, P. and Shapiro, G. (2009) A phase I study with neratinib (HKI-272), an irreversible pan ErbB receptor tyrosine kinase inhibitor, in patients with solid tumors. *Clin Cancer Res* 15: 2552–2558.

Yin, W., Jiang, Y., Shen, Z., Shao, Z. and Lu, J. (2011) Trastuzumab in the adjuvant treatment of HER2-positive early breast cancer patients: a meta-analysis of published randomized controlled trials. *PLoS One* 6: e21030.