

Emerging treatments for HER2-positive early-stage breast cancer: focus on neratinib

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Abstract: Over the last decades, a better understanding of breast cancer heterogeneity provided tools for a biologically based personalization of anticancer treatments. In particular, the overexpression of the human epidermal growth factor receptor 2 (HER2) by tumor cells provided a specific target in these HER2-positive tumors. The development of the monoclonal antibody trastuzumab, and its approval in 1998 for the treatment of patients with metastatic disease, radically changed the natural history of this aggressive subtype of breast cancer. These findings provided strong support for the continuous research in targeting the HER2 pathway and implementing the development of new anti-HER2 targeted agents. Besides trastuzumab, a series of other anti-HER2 agents have been developed and are currently being explored for the treatment of breast cancer patients, including those diagnosed with early-stage disease. Among these agents, neratinib, an oral tyrosine kinase inhibitor that irreversibly inhibits HER1, HER2, and HER4 at the intracellular level, has shown promising results, including when administered to patients previously exposed to trastuzumab-based treatment. This article aims to review the available data on the role of the HER2 pathway in breast cancer and on the different targeted agents that have been studied or are currently under development for the treatment of patients with early-stage HER2-positive disease with a particular focus on neratinib.

Keywords: breast cancer, HER2-positive, neratinib, early-stage, targeted therapy

Introduction

Despite early diagnosis and the availability of more active treatments, breast cancer remains a major public health problem.¹ To date, early-stage breast cancer is treated with curative intent by surgery with or without adjuvant treatments, including chemotherapy, endocrine therapy, targeted agents, and radiotherapy.²⁻⁴ However, ~20%–30% of women will relapse, despite optimal adjuvant therapies.^{5,6} Furthermore, these treatments and, particularly, chemotherapy are associated with significant toxicities that can alter the short- and long-term quality of life of cancer survivors.⁷ This underlies an unmet need to implement more effective therapies with acceptable toxicity profile for breast cancer patients with early-stage disease.

Over the last decades, a better understanding of breast cancer heterogeneity provided tools for a biologically based personalization of treatments.⁸ In particular, the overexpression of the human epidermal growth factor receptor 2 (HER2) by tumor cells is related to a distinctive molecular signature as compared to HER2-negative tumors.^{9,10} Indeed, HER2-positive breast cancers are poorly differentiated and characterized by having high grade, high rates of cell proliferation and lymph node involvement with a relative resistance to chemotherapies, and a greater likelihood of developing distant metastases.⁸ Interestingly, the expression of the HER2 protein in tumor cells provided a specific target in these HER2-positive tumors. The development of the

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monoclonal antibody trastuzumab and its approval in 1998 for the treatment of patients with metastatic disease radically changed the natural history of this subtype of breast cancer.^{11,12} These findings provided strong support for the continuous research in targeting the HER2 pathway and implementing the development of anti-HER2 targeted agents not only in the metastatic setting but also in the early setting.¹³ Chemotherapy plus 1 year of trastuzumab is the current standard of care for patients with early-stage HER2-positive breast cancer.²⁻⁴ Besides trastuzumab, a series of other anti-HER2 agents have been developed and are currently being explored for the treatment of breast cancer patients, including those diagnosed with early-stage disease. This article aims to review the available data on the role of the HER2 pathway in breast cancer and on the different targeted agents that have been studied or are currently under development for the treatment of patients with early-stage HER2-positive disease with a particular focus on neratinib.

HER2 pathway in breast cancer

The HER (ErbB) receptor family includes HER1 (ErbB1 or EGFR), HER2 (ErbB2, HER2/neu), HER3 (ErbB3), and HER4 (ErbB4) that act mechanistically through tyrosine kinases.¹⁴ The HER receptor comprises extracellular, transmembrane, and intracellular domains. Eleven ligands are known to bind to the HER receptors (HER1, 3, and 4), which expose the dimerization domain and facilitate homo- and heterodimerization. In contrast, HER2 does not have a known ligand and adopts a constantly exposed dimerization domain with potent formation of homodimers.¹⁵ Dimerization results in the activation of the phosphoinositide 3-kinase (PI3K) and of the mitogen-activated protein kinase downstream signaling pathways with subsequent promotion of cell survival and proliferation.¹⁶

Targeting HER2 may encounter de novo or acquired resistances that are not yet fully elucidated. The available knowledge of de novo resistance describes phosphatase and tensin homolog (PTEN) deficiencies, PI3K mutation, loss of p27, and HER2 cleavage or allosteric hindrance.¹⁷⁻²¹ Acquired resistance occurs secondary to the increased activation of insulin-like growth factor 1 (IGFR1) and c-Met with enhanced release of the HER ligands and activation of downstream signaling.²¹⁻²³ The presence of HER2 mutations may be another possible mechanism of resistance to anti-HER2 treatments.^{24,25} The potential development of resistance to anti-HER2 targeted agents represents an important rationale for implementing the dual targeting of the HER2 pathway.

Targeted agents for the treatment of HER2-positive early-stage breast cancer

The successful development of trastuzumab validated the treatment approach of targeting HER2. The following three other anti-HER2 targeted agents have been subsequently developed: lapatinib, pertuzumab, and T-DM1. Even more recently, a better understanding of the mechanisms of resistance to anti-HER2 therapy has led to the development of novel therapeutic agents such as neratinib.

Adjuvant setting

Trastuzumab is the first and so far the only targeted agent approved for the adjuvant treatment of patients with early-stage HER2-positive breast cancer.^{2,4} The benefit of adding trastuzumab to chemotherapy was evaluated in the following five main randomized studies: HERA,²⁶⁻²⁹ NSABP-B31 and NCCTG N9831,³⁰⁻³² BCIRG 006,^{33,34} and FNCLCC-PACS 04³⁵ trials. Among these studies, the joint analysis of two major North American Cooperative Group trials (NSABP B31 and NCCTG N9831) established the superiority of combining chemotherapy (doxorubicin plus cyclophosphamide followed by paclitaxel) with trastuzumab over the arms that did not contain the anti-HER2 agent, with the largest benefit shown with concurrent administration of taxanes and trastuzumab.³⁶ Although with a more strict patient selection and close cardiac assessment the incidence of trastuzumab-related cardiac events has been reduced over the past years, cardiotoxicity remains a significant problem in clinical practice.³⁷ Hence, trastuzumab was also developed and showed important efficacy results when combined with nonanthracycline-based regimens as in the BCIRG 006 trial.^{33,34} Therefore, in patients with cardiac risk factors, the use of this regimen can now be considered to overcome any potential cardiac side effects.³⁸ The HERA trial confirmed that the standard duration of adjuvant trastuzumab is 12 months.^{28,29} A Cochrane review that included eight randomized control trials with 11,991 patients with HER2-positive tumors confirmed the superiority of trastuzumab-containing regimens in terms of both disease-free survival (DFS; hazard ratio [HR] 0.60; 95% confidence intervals [CIs] 0.50–0.71; $P < 0.0001$) and overall survival (OS; HR 0.66; 95% CI 0.57–0.77; $P < 0.00001$).³⁹ Trastuzumab administration was associated with a significant increased risk of congestive heart failure (risk ratio [RR] 5.11; 90% CI 3.0–8.7; $P < 0.00001$) and left ventricular ejection fraction decline (RR 1.8; 90% CI 1.4–2.5; $P = 0.0008$).³⁹ Nevertheless, in more recent studies, with a strict patient selection and close cardiac assessment, the risk of cardiac events

with the use of trastuzumab-based regimens has decreased significantly.³⁷ As shown in the HERA trial, the majority of cardiac events occurred during treatment and were reversible in ~80% of the cases.⁴⁰

If trastuzumab is a monoclonal antibody that interferes with HER2 outside of the cell, lapatinib is an oral tyrosine kinase inhibitor that reversibly inhibits both HER1 and HER2 at the intracellular level.⁴¹ Lapatinib has been investigated in two trials of patients with HER2-positive early-stage breast cancer.¹³

The TEACH study compared lapatinib with placebo in 3,147 trastuzumab-naïve patients and failed to show a statistically significant difference in DFS (HR 0.83; 95% CI 0.7–1.0; $P=0.053$).^{42,43} The central review of HER2 status revealed that only 79% of the randomized patients were actually HER2 positive. In this group of patients, an improved DFS was observed with the use of lapatinib (HR 0.82; 95% CI 0.7–1.0; $P=0.04$). Treatment-related grade 3–4 toxicities included diarrhea (6%), rash (5%), and hepatobiliary disorders (2%). Of note, in this trial, the level of HER2 positivity was specified according to the cutoff used for trastuzumab approval.

The ALTT0 study investigated the efficacy of lapatinib alone, the sequence of trastuzumab followed by lapatinib, or the dual anti-HER2 blockade (lapatinib plus trastuzumab) as compared to standard trastuzumab for 1 year in 8,381 patients.⁴⁴ At interim analysis, due to futility in demonstrating noninferiority of lapatinib versus trastuzumab, the lapatinib arm was closed and trastuzumab was offered to disease-free patients. As compared to trastuzumab alone, dual anti-HER2 blockade with lapatinib and trastuzumab was not associated with a significant improved DFS (HR 0.84; 95% CI 0.7–1.02; $P=0.048$) at the 0.025 significance level foreseen in the statistical analysis plan of the study. As compared with patients treated with trastuzumab, those who underwent lapatinib experienced more diarrhea, cutaneous rash, and hepatic toxicity.⁴⁴

Pertuzumab is a monoclonal antibody that inhibits the HER2–HER3 heterodimerization.⁴⁵ Pertuzumab had been demonstrated to significantly improve OS by 15.7 months in the metastatic setting when added to a regimen containing docetaxel plus trastuzumab.⁴⁶ In the adjuvant setting, the Phase III APHINITY trial (NCT01358877) is currently evaluating the benefit of its addition to trastuzumab. A press release on March 1, 2017, confirmed a significant improvement in invasive DFS (iDFS) with the addition of pertuzumab to trastuzumab in patients with early-stage HER2-positive breast cancer. Full results of the study are expected to be

reported at the 2017 American Society of Clinical Oncology (ASCO) Annual Meeting in June.

T-DM1 is an anti-HER2 antibody–drug conjugate that combines emtansine (a cytotoxic agent) to trastuzumab to further increase the toxicity of the monoclonal antibody by targeting its activity directly to cancer cells.⁴⁷ T-DM1 administration improves progression-free survival and OS in the metastatic setting in case of trastuzumab-resistant HER2-positive breast cancer.^{48,49} So far, no data are yet available in early-stage breast cancer, but the compound is actually being investigated in three Phase III trials.¹³ The KATHERINE study is comparing 14 cycles of adjuvant T-DM1 with trastuzumab in patients with residual disease following neoadjuvant chemotherapy combined with trastuzumab (NCT01772472). The KAITLIN trial is comparing the combination of T-DM1 and pertuzumab with a taxane after surgery and anthracycline-based chemotherapy (NCT01966471). Finally, the ATEMPT trial is comparing T-DM1 alone with paclitaxel plus trastuzumab followed by trastuzumab in stage I HER2-positive breast cancer (NCT01853748).

Neoadjuvant setting

The use of trastuzumab in the neoadjuvant setting of HER2-positive early-stage breast cancer has revolutionized the management of these patients leading to the clinical development of new anti-HER2 targeted therapies in this setting.

The first of the large Phase III trials that supported the combination of trastuzumab and anthracycline-based chemotherapy was the NOAH trial.^{50,51} The study included 235 patients with locally advanced breast cancers and confirmed a beneficial effect of adding trastuzumab to chemotherapy in improving both pathological complete response (pCR) rate and survival outcomes. Subsequent studies confirmed that the addition of trastuzumab as part of neoadjuvant therapy significantly increases pCR with no additional toxicity and is currently the standard of care in this setting.⁵² As shown in the ACOSOG Z1041 trial, trastuzumab should be administered concurrently to taxane-based chemotherapy, while the administration with anthracyclines should be avoided.⁵³ More recently, the HannaH trial validated the subcutaneous route of administration of trastuzumab as an alternative option.^{54–56}

The combination of lapatinib and trastuzumab has been evaluated in multiple randomized trials.⁵⁷ When combining the results from the six randomized studies that investigated this combination, the dual blockade with trastuzumab and lapatinib was particularly active only in patients with hormone receptor-negative disease and treated with

taxane chemotherapy. Nevertheless, the increased pCR rates with the use of this combination did not translate in survival benefit.⁵⁸

Pertuzumab has been evaluated in two Phase II neoadjuvant trials (ie, NeoSphere and TRYPHAENA studies) in which patients received a combination of trastuzumab and chemotherapy with or without pertuzumab.^{59–61} As compared to the use of chemotherapy and trastuzumab alone, dual anti-HER2 blockade with pertuzumab and trastuzumab was associated with a significant improvement in pCR rate and a trend toward increased survival. Safety was confirmed with low rates of cardiac dysfunction in the investigational arms.

Neratinib in HER2-positive early-stage breast cancer

Neratinib is an oral tyrosine kinase inhibitor that irreversibly inhibits HER1, HER2, and HER4 at the intracellular level.^{62,63} This small molecule has shown promising results in both the metastatic and early settings including when administered to patients who have previously received trastuzumab-based treatment.^{62,63}

Adjuvant setting

Up to one-third of patients with HER2-positive early-stage breast cancer develop recurrent disease even when adequately treated.^{29,32} Hence, attempts to escalate the standard treatment with chemotherapy and 1 year of trastuzumab should be considered of crucial importance for this subgroup of patients. Among these attempts, extending the duration of trastuzumab up to 2 years and the combination of trastuzumab and bevacizumab or lapatinib have failed.¹³ The ExteNET trial challenged another way to escalate treatment in this subgroup of patients by using neratinib at the completion of standard chemotherapy and 1 year of trastuzumab.⁶⁴

The ExteNET study was a randomized, double-blind, placebo-controlled, Phase III trial that included a total of 2,840 patients with HER2-positive stage I–III breast cancer who had previously completed 1 year of adjuvant trastuzumab. Eligible patients were randomized to an additional 12 months of treatment with oral neratinib (240 mg/day) or matching placebo. Randomization was performed in a 1:1 ratio and was generated with permuted blocks stratified by hormonal receptor status, nodal status, and trastuzumab mode of administration. A total of 31.5% of patients had tumors of ≤ 2 cm, 24% of them had node-negative disease, and 57% of the patients had hormone receptor-positive disease. After a median follow-up of 2 years, DFS was 93.9% in the neratinib arm and 91.6% in the placebo arm,

favoring the use of tyrosine kinase inhibitor (HR 0.67; 95% CI 0.5–0.91; $P=0.0091$). The cumulative incidence of central nervous system recurrence was not different in the two arms (0.91% in the neratinib arm and 1.25% in the placebo arm, $P=0.44$), but longer follow-up is foreseen for acquiring better insight on this endpoint. The most common grade 3–4 adverse events in the neratinib arm included diarrhea (40%), vomiting (3%), and nausea (2%). Furthermore, patients reported to have an altered quality of life in the first month of treatment by neratinib followed by recovery toward baseline levels afterward.

Of note, in comparison to the HERA trial, which failed to show a DFS improvement by increasing anti-HER2 treatment duration from 1 to 2 years, the ExteNET study enrolled patients with lower incidence of lymph node involvement (24 vs 32%, respectively) and administered more commonly trastuzumab with concurrent chemotherapy (62 vs 0%, respectively) and more frequently the combination of anthracyclines and taxanes as adjuvant chemotherapy (68 vs 26%, respectively). It is noteworthy also the presence of 4.5 months between the end of trastuzumab and the initiation of neratinib in the ExteNET trial, while this delay was not present in the HERA trial for trastuzumab initiation after chemotherapy (89 days). The authors attributed the positive results of the ExteNET trial to the oncogenic addiction of HER2-positive tumors to the HER2 pathway with the recurrence of breast cancer being secondary to an acquired resistance to trastuzumab that can lead to a possible reactivation of the HER2 pathway after continuous inhibition. Adding neratinib would overcome this resistance especially in the absence of cross-resistance with trastuzumab. Interestingly, a prespecified subgroup analysis of iDFS according to hormone receptor status showed that neratinib provided greater benefit to patients with hormone receptor-positive breast cancer (HR 0.51; 95% CI 0.33–0.77; $P=0.0013$) than to those with hormone receptor-negative tumors (HR 0.93; 95% CI 0.6–1.43; $P=0.74$; P for interaction = 0.054). Longer follow-up data from the study are awaited to better understand the potential role of neratinib in this setting.

In terms of toxicity, diarrhea is the most important side effect observed with the administration of neratinib (in the ExteNET trial, 55 and 40% of patients developed grade 1–2 and grade 3–4 diarrhea, respectively).⁶⁴ To overcome this issue, intensive loperamide prophylaxis during the first month of treatment has been shown to effectively reduce the occurrence of diarrhea and improves the overall tolerability to the drug.⁶⁵ An open-label Phase II study is currently assessing the incidence and severity of diarrhea when routine use of

intensive loperamide prophylaxis is administered during the first month of neratinib therapy (NCT02400476).

Neoadjuvant setting

The I-SPY 2 TRIAL (Investigator of Serial Studies to Predict Your Therapeutic Response through Imaging and Molecular Analysis 2) is a Phase II “platform” trial with a backbone of standard therapy to which multiple experimental compounds can be added and compared with a common control regimen.⁶⁶ Patients with stage II–III breast cancer were included in the study with the only exception of women with hormone receptor-positive and low-risk tumors as assessed by the 70-gene assay. For the purpose of randomization, eligible patients were categorized into eight different subtypes according to HER2 status, hormone receptor status, and genomic risk score. All patients received a neoadjuvant treatment with 12 cycles of weekly paclitaxel in the control group or in combination with one of the several experimental agents. Before surgery, all patients in all treatment arms received four cycles of doxorubicin and cyclophosphamide. The assignment to the experimental group was performed through an adaptive randomization procedure. With this approach, new patients entering the trial are more likely to be randomized to receive the compound that showed to be active for their specific tumor subtype. At the same time, this type of trial design allows a more rapid selection of promising regimens to be then developed in Phase III trials.⁶⁶

One of the experimental therapy groups in the I-SPY 2 trial evaluated the addition of neratinib.⁶⁷ In this analysis, both patients with HER2-positive and -negative diseases could be included. Patients in the experimental arm received neratinib in addition to chemotherapy (n=115), while patients in the control group (n=78) received weekly paclitaxel alone if HER2 negative or with trastuzumab if HER2 positive. Neratinib reached the predetermined threshold of efficacy with regard to patients with HER2-positive/hormone receptor-negative disease. In these patients, the estimated rate of pCR was 56% in the neratinib group and 33% in the control group; the resulting probability that neratinib was superior to standard therapy was 95% and the probability of success in a Phase III trial including 300 patients was 79%. In patients with HER2-positive/hormone receptor-positive tumors, neratinib also showed some evidence of superior activity. In these patients, the estimated rate of pCR was 30% in the neratinib group and 17% in the control group. This translated in a 91% probability of superiority of neratinib over standard therapy and a 65% predicted probability of success in a Phase III trial. When considering all patients with HER2-positive disease

irrespective of hormone receptor status, the estimated rate of pCR was 39% in the neratinib group and 23% in the control group. The resulting probability that neratinib was superior to standard therapy was 95%, and the probability of success in a Phase III trial including 300 patients was 73%. There was limited activity of neratinib in patients with HER2-negative disease independently of their hormonal receptor status, and the adaptive randomization algorithm stopped assigning these patients to receive the anti-HER2 tyrosine kinase inhibitor. Grade 3–4 treatment-related adverse events were mostly limited to diarrhea (38%) and were mitigated by dose reductions and/or symptomatic management. Results on survival from the I-SPY 2 trial are not mature yet.⁶⁰

More recently, neratinib was also evaluated in the neoadjuvant NSABP FB 7 Phase II trial (NCT01008150).⁶⁸ This study had a backbone of weekly paclitaxel followed by doxorubicin and cyclophosphamide. It included three arms consisting of trastuzumab, neratinib, or both added to paclitaxel. Preliminary results of this study have been reported at the San Antonio Annual Meeting in 2015. Overall, pCR rate was higher for the combination of trastuzumab and neratinib (50.0%) in comparison to trastuzumab alone (38.1%) and neratinib alone (33.3%). The latter two results conflict with the I-SPY 2 trial in which neratinib was expected to be superior to trastuzumab. Final results from this study are awaited, including a longer follow-up to investigate possible survival difference among treatments.

Ongoing trials with neratinib in HER2-positive early-stage breast cancer

We conducted an electronic research of the registered trials until March 2017 using www.clinicaltrials.gov to identify the ongoing trials evaluating the role of neratinib in breast cancer. Our research yielded 23 registered trials, of which five trials included patients with early-stage breast cancer (Table 1).

Conclusion and future perspectives

More than 10 years have passed since the addition of 1 year trastuzumab to chemotherapy in the treatment of patients with HER2-positive early-stage breast cancer.^{2–4} Several efforts in trying to de-escalate or to escalate this approach have been investigated over the past years.^{13,69} For a specific subgroup of patients with node-negative and small tumors, de-escalating chemotherapy burden with the use of anthracycline-free regimen (weekly paclitaxel for 12 cycles) proved to be effective and can now be considered an option.⁷⁰ In contrast, as further highlighted by long-term outcome results from the pivotal trastuzumab trials,^{29,32} ~25%–30% of women with

Table 1 Ongoing clinical trials with neratinib in early-stage breast cancer according to www.clinicaltrials.gov entries until March 2017

NCT number	Phase	Study title	Subjects, n	Status
NCT01008150	II	Phase II randomized trial evaluating neoadjuvant therapy with neratinib and/or trastuzumab followed by postoperative trastuzumab in women with locally advanced HER2-positive breast cancer	141	Active but not recruiting
NCT02400476	II	A study looking at the incidence and severity of diarrhea in patients with early-stage HER2-positive breast cancer treated with neratinib and loperamide	240	Active and recruiting
NCT00878709	III	Study evaluating the effects of neratinib after adjuvant trastuzumab in women with early stage breast cancer (ExteNET)	2,840	Active but not recruiting
NCT01042379	II	I-SPY 2 TRIAL: neoadjuvant and personalized adaptive novel agents to treat breast cancer (I-SPY 2)	1,920	Active and recruiting
NCT01008150	II	Phase II randomized trial evaluating neoadjuvant therapy with neratinib and/or trastuzumab followed by postoperative trastuzumab in women with locally advanced HER2-positive breast cancer	141	Active but not recruiting

Abbreviation: HER2, human epidermal growth factor receptor 2.

HER2-positive early-stage breast cancer still relapse at 10 years. Further research efforts for trying to improve these outcomes should be considered as a research priority. Neratinib has shown promising results in this setting demonstrating to be an active molecule in both the adjuvant and neoadjuvant settings (Table 2). However, long-term follow-up data from the available trials as well as further studies are needed to better clarify its role in patients with HER2-positive early-stage breast cancer treated with trastuzumab-based therapy.

With the availability in the near future of another possible escalating strategy with the dual anti-HER2 blockade trastuzumab plus pertuzumab, the clinical management of patients with HER2-positive early-stage breast cancer may become

even more challenging. In this scenario, translational research efforts should be prioritized in order to better identify which patients are not cured with chemotherapy plus trastuzumab. Predictive biomarkers beyond HER2 are also needed to better guide the preferred treatment option in these cases. So far, the main efforts in this field have focused on the potential role of HER2 receptor, PI3K, c-MYC expression, and the host immune system.

Several studies investigated the prognostic and/or predictive values of the *HER2* gene polymorphisms,⁷¹ specific domains and alterations in the HER2 receptor,^{21,72–74} and soluble HER2 levels.^{75,76} However, no definitive conclusions can be drawn on this regard, especially for their predictive role in the adjuvant setting.

Table 2 Available clinical trials with the use of neratinib in early-stage breast cancer

Trial	Study design	Setting/ population	Number of patients	Treatment arm	Main results
ExteNET ⁶⁴	Phase III	Adjuvant setting/ stage I–III breast HER2-positive	1,420	CT + trastuzumab for 1 year + placebo for another year	2-year IDFS: 91.6%
			1,420	CT + trastuzumab for 1 year + neratinib for another year	2-year IDFS: 93.9%
I-SPY 2 ⁶⁷	Phase II	Neoadjuvant setting/ stage II/III HER2-positive or -negative ^a	115	CT + neratinib	pCR in HER2-positive/ER-negative: 56% pCR in HER2-positive/ER-positive: 30% pCR in HER2-positive: 39%
			78	Paclitaxel alone in HER2-negative and with trastuzumab in HER2-positive	pCR in HER2-positive/ER-negative: 33% pCR in HER2-positive/ER-positive: 17% pCR in HER2-positive: 23%
NSABP FB 7 ⁶⁸	Phase II	Neoadjuvant setting/ stage IIB/IIIC HER2-positive	126	Paclitaxel + trastuzumab followed by doxorubicin and cyclophosphamide	pCR: 38%
				Paclitaxel + neratinib followed by doxorubicin and cyclophosphamide	pCR: 33%
				Paclitaxel + neratinib + trastuzumab followed by doxorubicin and cyclophosphamide	pCR: 50%

Note: ^aThere was limited activity of neratinib in patients with HER2-negative disease independently of their hormonal receptor status, and the adaptive randomization algorithm stopped assigning these patients to receive the anti-HER2 tyrosine kinase inhibitor.

Abbreviations: CT, chemotherapy; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IDFS, invasive disease-free survival; pCR, pathological complete response.

Alterations that lead to constitutive activation of the PI3K pathway, including PI3K catalytic subunit alpha (*PIK3CA*) mutation and low *PTEN* expression, seem to be mediators of trastuzumab resistance.⁷⁷ Several studies in both the metastatic^{78–80} and neoadjuvant^{81–83} settings appear to be in the same line of the findings from preclinical experiments. However, the available data in the adjuvant setting did not show any predictive role for *PIK3CA* or *PTEN*.^{84–87}

The *MYC* oncoprotein acts as a downstream target of the proliferative signals driven by HER2 and has a potential role as predictor of response to anti-HER2-targeted therapies.⁸⁸ Although preliminary evidence suggested that *MYC* gene copy number abnormalities could be associated with additional benefit to trastuzumab even in the adjuvant setting,⁸⁹ subsequent research did not confirm the potential predictive role of *MYC* protein expression.⁹⁰

Preclinical and clinical studies showed that the immune system gives a substantial contribution to the therapeutic effects of trastuzumab.^{91,92} Several studies have recently assessed the prognostic and predictive value of tumor-infiltrating lymphocytes (TILs).^{93–95} In the neoadjuvant setting, a recent large meta-analysis confirmed the association between pre-treatment TIL levels and the probability of achieving pCR in patients with HER2-positive early-stage breast cancer treated with anti-HER2 agents.⁹⁶ However, no association between TILs and pCR rates according to the type of administered anti-HER2 therapy (single or dual blockade) was observed.⁹⁶ In the adjuvant setting, data from the FinHER and NCCTG N9831 trials reported conflicting results on the role of TILs in predicting the benefit from trastuzumab.^{97,98} Hence, the role of TILs as contributors of the immune effects of trastuzumab antitumor activity remains controversial and needs to be further evaluated in patients with HER2-positive early-stage breast cancer.⁹⁹ Immune gene signatures may be useful in this regard, by giving complementary information and more in depth insights on the immune functional status.⁹⁹

The conflicting data in this field highlight the urgent need for further translational efforts in this setting from the clinical trials that have investigated the two anti-HER2 agents (ie, neratinib and pertuzumab) that proved to be of added benefit in patients already receiving standard chemotherapy plus trastuzumab for 1 year.

Acknowledgments

Matteo Lambertini acknowledges the support from the European Society for Medical Oncology (ESMO) for a Translational Research Fellowship at Institut Jules Bordet. EdA and ML are co-last authors.

Disclosure

EdA received honoraria from Roche and travel grants from Roche and GlaxoSmithKline outside the submitted work. His institution has received research grants from Roche. The other authors report no conflicts of interest in this work.

References

- Ginsburg O, Bray F, Coleman MP, et al. The global burden of women's cancers: a grand challenge in global health. *Lancet*. 2017;389(10071):847–860.
- Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26(suppl 5):v8–v30.
- Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol*. 2015;26(8):1533–1546.
- Denduluri N, Somerfield MR, Eisen A, et al. Selection of optimal adjuvant chemotherapy regimens for human epidermal growth factor receptor 2 (HER2)-negative and adjuvant targeted therapy for HER2-positive breast cancers: an American Society of Clinical Oncology Guideline Adaptation of the Cancer Care Ontario Clinical Practice Guideline. *J Clin Oncol*. 2016;34(20):2416–2427.
- Cardoso F, Costa A, Senkus E, et al. 3rd ESO-ESMO international consensus guidelines for Advanced Breast Cancer (ABC 3). *Breast*. 2017;31:244–259.
- Cardoso F, Costa A, Senkus E, et al. 3rd ESO-ESMO international consensus guidelines for Advanced Breast Cancer (ABC 3). *Ann Oncol*. 2017;28(1):16–33.
- Lambertini M, Del Mastro L, Pescio MC, et al. Cancer and fertility preservation: international recommendations from an expert meeting. *BMC Med*. 2016;14(1):1.
- Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747–752.
- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177–182.
- Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989;244(4905):707–712.
- Leyland-Jones B. Trastuzumab: hopes and realities. *Lancet Oncol*. 2002;3(3):137–144.
- Del Mastro L, Lambertini M, Bighin C, et al. Trastuzumab as first-line therapy in HER2-positive metastatic breast cancer patients. *Expert Rev Anticancer Ther*. 2012;12(11):1391–1405.
- Lambertini M, Pondé NF, Solinas C, de Azambuja E. Adjuvant trastuzumab: a 10-year overview of its benefit. *Expert Rev Anticancer Ther*. 2017;17(1):61–74.
- Baselga J, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat Rev Cancer*. 2009;9(7):463–475.
- Klapper LN, Glathe S, Vaisman N, et al. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc Natl Acad Sci USA*. 1999;96(9):4995–5000.
- Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene*. 2007;26(45):6469–6487.
- Nagata Y, Lan K-H, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell*. 2004;6(2):117–127.
- Nahta R, Takahashi T, Ueno NT, Hung M-C, Esteva FJ. P27(kip1) down-regulation is associated with trastuzumab resistance in breast cancer cells. *Cancer Res*. 2004;64(11):3981–3986.

19. Nagy P, Friedländer E, Tanner M, et al. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res.* 2005;65(2):473–482.
20. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res.* 2005;65(7):2554–2559.
21. Scaltriti M, Rojo F, Ocaña A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst.* 2007;99(8):628–638.
22. Shattuck DL, Miller JK, Carraway KL, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res.* 2008;68(5):1471–1477.
23. Feldinger K, Generali D, Kramer-Marek G, et al. ADAM10 mediates trastuzumab resistance and is correlated with survival in HER2 positive breast cancer. *Oncotarget.* 2014;5(16):6633–6646.
24. Park YH, Shin H-T, Jung HH, et al. Role of HER2 mutations in refractory metastatic breast cancers: targeted sequencing results in patients with refractory breast cancer. *Oncotarget.* 2015;6(31):32027–32038.
25. Hanker AB, Red Brewer M, Sheehan JH, et al. An acquired HER2 T798I gatekeeper mutation induces resistance to neratinib in a patient with HER2 mutant-driven breast cancer. *Cancer Discov.* 2017;7(6):575–585.
26. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med.* 2005;353(16):1659–1672.
27. Gianni L, Dafni U, Gelber RD, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol.* 2011;12(3):236–244.
28. Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, et al. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. *Lancet.* 2013;382(9897):1021–1028.
29. Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet.* 2017;389(10075):1195–1205.
30. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med.* 2005;353(16):1673–1684.
31. Perez EA, Romond EH, Suman VJ, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clin Oncol.* 2011;29(25):3366–3373.
32. Perez EA, Romond EH, Suman VJ, et al. 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.* 2014;32(33):3744–3752.
33. Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med.* 2011;365(14):1273–1283.
34. Slamon DJ, Eiermann W, Robert NJ, et al. Ten year follow-up of the BCIRG-006 trial comparing doxorubicin plus cyclophosphamide followed by docetaxel (AC@T) with doxorubicin plus cyclophosphamide followed by docetaxel and trastuzumab (AC@TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2+ early breast cancer patients. In: San Antonio Breast Cancer Symposium 2015; Abstract:SS-04.
35. Spielmann M, Roché H, Delozier T, et al. Trastuzumab for patients with axillary-node-positive breast cancer: results of the FNCLCC-PACS 04 trial. *J Clin Oncol.* 2009;27(36):6129–6134.
36. Perez EA, Suman VJ, Davidson NE, et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. *J Clin Oncol.* 2011;29(34):4491–4497.
37. Pondé NF, Lambertini M, de Azambuja E. Twenty years of anti-HER2 therapy-associated cardiotoxicity. *ESMO Open.* 2016;1(4):e000073.
38. Pal SK, Childs BH, Pegram M. Emergence of nonanthracycline regimens in the adjuvant treatment of breast cancer. *Breast Cancer Res Treat.* 2010;119(1):25–32.
39. Moja L, Tagliabue L, Balduzzi S, et al. Trastuzumab containing regimens for early breast cancer. *Cochrane Database Syst Rev.* 2012;(4):CD006243.
40. de Azambuja E, Procter MJ, van Veldhuisen DJ, et al. Trastuzumab-associated cardiac events at 8 years of median follow-up in the Herceptin Adjuvant trial (BIG 1-01). *J Clin Oncol.* 2014;32(20):2159–2165.
41. Zhang X, Munster PN. New protein kinase inhibitors in breast cancer: afatinib and neratinib. *Expert Opin Pharmacother.* 2014;15(9):1277–1288.
42. Goss PE, Smith IE, O'Shaughnessy J, et al. Adjuvant lapatinib for women with early-stage HER2-positive breast cancer: a randomised, controlled, phase 3 trial. *Lancet Oncol.* 2013;14(1):88–96.
43. Strasser-Weippl K, Horick N, Smith IE, et al. Identification of early breast cancer patient cohorts who may benefit from lapatinib therapy. *Eur J Cancer.* 2016;56:85–92.
44. Piccart-Gebhart M, Holmes E, Baselga J, et al. Adjuvant lapatinib and trastuzumab for early human epidermal growth factor receptor 2-positive breast cancer: results from the randomised phase III adjuvant lapatinib and/or trastuzumab treatment optimization trial. *J Clin Oncol.* 2016;34(10):1034–1042.
45. Gao J, Swain SM. Pertuzumab for the treatment of breast cancer: a safety review. *Expert Opin Drug Saf.* 2016;15(6):853–863.
46. Swain SM, Baselga J, Kim S-B, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med.* 2015;372(8):724–734.
47. Martínez MT, Pérez-Fidalgo JA, Martín-Martorell P, et al. Treatment of HER2 positive advanced breast cancer with T-DM1: a review of the literature. *Crit Rev Oncol Hematol.* 2016;97:96–106.
48. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med.* 2012;367(19):1783–1791.
49. Krop IE, Kim S-B, González-Martín A, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2014;15(7):689–699.
50. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet.* 2010;375(9712):377–384.
51. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet Oncol.* 2014;15(6):640–647.
52. Valachis A, Mauri D, Polyzos NP, Chlouverakis G, Mavroudis D, Georgoulas V. Trastuzumab combined to neoadjuvant chemotherapy in patients with HER2-positive breast cancer: a systematic review and meta-analysis. *Breast.* 2011;20(6):485–490.
53. Buzdar AU, Suman VJ, Meric-Bernstam F, et al. Fluorouracil, epirubicin, and cyclophosphamide (FEC-75) followed by paclitaxel plus trastuzumab versus paclitaxel plus trastuzumab followed by FEC-75 plus trastuzumab as neoadjuvant treatment for patients with HER2-positive breast cancer (Z1041): a randomised, controlled, phase 3 trial. *Lancet Oncol.* 2013;14(13):1317–1325.
54. Ismael G, Hegg R, Muehlbauer S, et al. Subcutaneous versus intravenous administration of (neo)adjuvant trastuzumab in patients with HER2-positive, clinical stage I-III breast cancer (HannaH study): a phase 3, open-label, multicentre, randomised trial. *Lancet Oncol.* 2012;13(9):869–878.
55. Jackisch C, Kim S-B, Semiglazov V, et al. Subcutaneous versus intravenous formulation of trastuzumab for HER2-positive early breast cancer: updated results from the phase III HannaH study. *Ann Oncol.* 2015;26(2):320–325.

56. Jackisch C, Hegg R, Stroyakovskiy D, et al. HannaH phase III randomised study: association of total pathological complete response with event-free survival in HER2-positive early breast cancer treated with neoadjuvant-adjuvant trastuzumab after 2 years of treatment-free follow-up. *Eur J Cancer*. 2016;62:62–75.
57. Clavarezza M, Puntoni M, Gennari A, et al. Dual block with lapatinib and trastuzumab versus single-agent trastuzumab combined with chemotherapy as neoadjuvant treatment of HER2-positive breast cancer: a meta-analysis of randomized trials. *Clin Cancer Res*. 2016;22(18):4594–4603.
58. de Azambuja E, Holmes AP, Piccart-Gebhart M, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTO): survival outcomes of a randomised, open-label, multicentre, phase 3 trial and their association with pathological complete response. *Lancet Oncol*. 2014;15(10):1137–1146.
59. Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). *Ann Oncol*. 2013;24(9):2278–2284.
60. Gianni L, Pienkowski T, Im Y-H, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2012;13(1):25–32.
61. Gianni L, Pienkowski T, Im Y-H, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *Lancet Oncol*. 2016;17(6):791–800.
62. Kourie HR, Chaix M, Gombos A, Aftimos P, Awada A. Pharmacodynamics, pharmacokinetics and clinical efficacy of neratinib in HER2-positive breast cancer and breast cancer with HER2 mutations. *Expert Opin Drug Metab Toxicol*. 2016;12(8):947–957.
63. Tiwari SR, Mishra P, Abraham J. Neratinib, a novel HER2-targeted tyrosine kinase inhibitor. *Clin Breast Cancer*. 2016;16(5):344–348.
64. Chan A, Delalogue S, Holmes FA, 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. *Lancet Oncol*. 2016;17(3):367–377.
65. Andreyev J, Ross P, Donnellan C, et al. Guidance on the management of diarrhoea during cancer chemotherapy. *Lancet Oncol*. 2014;15(10):e447–e460.
66. Rugo HS, Olopade OI, DeMichele A, et al. Adaptive randomization of veliparib-carboplatin treatment in breast cancer. *N Engl J Med*. 2016;375(1):23–34.
67. Park JW, Liu MC, Yee D, et al. Adaptive randomization of neratinib in early breast cancer. *N Engl J Med*. 2016;375(1):11–22.
68. Jacobs SA, Robidoux A, Garcia JM, et al. 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 (NþT) followed by doxorubicin and cyclophosphamide (AC) with postoperative T in women with locally advanced HER2-positive breast cancer. In: San Antonio Breast Cancer Symposium 2015; Abstract PG5-04.
69. Joensuu H. Escalating and de-escalating treatment in HER2-positive early breast cancer. *Cancer Treat Rev*. 2017;52:1–11.
70. Tolaney SM, Barry WT, Dang CT, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. *N Engl J Med*. 2015;372(2):134–141.
71. Han X, Diao L, Xu Y, et al. Association between the HER2 Ile655Val polymorphism and response to trastuzumab in women with operable primary breast cancer. *Ann Oncol*. 2014;25(6):1158–1164.
72. Parra-Palau JL, Morancho B, Peg V, et al. Effect of p95HER2/611CTF on the response to trastuzumab and chemotherapy. *J Natl Cancer Inst*. 2014;106(11):dju291.
73. Montemurro F, Prat A, Rossi V, et al. Potential biomarkers of long-term benefit from single-agent trastuzumab or lapatinib in HER2-positive metastatic breast cancer. *Mol Oncol*. 2014;8(1):20–26.
74. Carvajal-Hausdorf DE, Schalper KA, Puzsai L, et al. Measurement of domain-specific HER2 (ERBB2) expression may classify benefit from trastuzumab in breast cancer. *J Natl Cancer Inst*. 2015;107(8):dju136.
75. Lee SB, Lee JW, Yu JH, et al. Preoperative serum HER2 extracellular domain levels in primary invasive breast cancer. *BMC Cancer*. 2014;14:929.
76. Moreno-Aspitia A, Hillman DW, Dyar SH, et al. Soluble human epidermal growth factor receptor 2 (HER2) levels in patients with HER2-positive breast cancer receiving chemotherapy with or without trastuzumab: results from North Central Cancer Treatment Group adjuvant trial N9831. *Cancer*. 2013;119(15):2675–2682.
77. Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007;12(4):395–402.
78. André F, O'Regan R, Ozguroglu M, et al. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol*. 2014;15(6):580–591.
79. Hurvitz SA, Andre F, Jiang Z, et al. Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1): a phase 3, randomised, double-blind, multicentre trial. *Lancet Oncol*. 2015;16(7):816–829.
80. André F, Hurvitz S, Fasolo A, et al. Molecular alterations and everolimus efficacy in human epidermal growth factor receptor 2-overexpressing metastatic breast cancers: combined exploratory biomarker analysis from BOLERO-1 and BOLERO-3. *J Clin Oncol*. 2016;34(18):2115–2124.
81. Ibrahim EM, Kazkaz GA, Al-Mansour MM, Al-Foheidi ME. The predictive and prognostic role of phosphatase phosphoinositol-3 (PI3) kinase (PIK3CA) mutation in HER2-positive breast cancer receiving HER2-targeted therapy: a meta-analysis. *Breast Cancer Res Treat*. 2015;152(3):463–476.
82. Loibl S, Darb-Esfahani S, Huober J, et al. Integrated analysis of PTEN and p4EBP1 protein expression as predictors for pCR in HER2-positive breast cancer. *Clin Cancer Res*. 2016;22(11):2675–2683.
83. Loibl S, Majewski I, Guarneri V, et al. PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. *Ann Oncol*. 2016;27(8):1519–1525.
84. Loi S, Michiels S, Lambrechts D, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. *J Natl Cancer Inst*. 2013;105(13):960–967.
85. Perez EA, Dueck AC, McCullough AE, et al. Impact of PTEN protein expression on benefit from adjuvant trastuzumab in early-stage human epidermal growth factor receptor 2-positive breast cancer in the North Central Cancer Treatment Group N9831 trial. *J Clin Oncol*. 2013;31(17):2115–2122.
86. Pogue-Geile KL, Song N, Jeong J-H, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. *J Clin Oncol*. 2015;33(12):1340–1347.
87. Stern HM, Gardner H, Burzykowski T, et al. PTEN loss is associated with worse outcome in HER2-amplified breast cancer patients but is not associated with trastuzumab resistance. *Clin Cancer Res*. 2015;21(9):2065–2074.
88. Chen Y, Olopade OI. MYC in breast tumor progression. *Expert Rev Anticancer Ther*. 2008;8(10):1689–1698.
89. Perez EA, Jenkins RB, Dueck AC, et al. C-MYC alterations and association with patient outcome in early-stage HER2-positive breast cancer from the north central cancer treatment group N9831 adjuvant trastuzumab trial. *J Clin Oncol*. 2011;29(6):651–659.

90. Dueck AC, Reinholz MM, Geiger XJ, et al. Impact of c-MYC protein expression on outcome of patients with early-stage HER2+ breast cancer treated with adjuvant trastuzumab NCCTG (alliance) N9831. *Clin Cancer Res.* 2013;19(20):5798–5807.
91. Bianchini G, Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. *Lancet Oncol.* 2014;15(2):e58–e68.
92. Knutson KL, Clynes R, Shreeder B, et al. Improved survival of HER2+ breast cancer patients treated with trastuzumab and chemotherapy is associated with host antibody immunity against the HER2 intracellular domain. *Cancer Res.* 2016;76(13):3702–3710.
93. Dieci MV, Mathieu MC, Guarneri V, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials. *Ann Oncol.* 2015;26(8):1698–1704.
94. Perez EA, Ballman KV, Tenner KS, et al. Association of stromal tumor-infiltrating lymphocytes with recurrence-free survival in the N9831 adjuvant trial in patients with early-stage HER2-positive breast cancer. *JAMA Oncol.* 2016;2(1):56–64.
95. Ingold Heppner B, Untch M, Denkert C, et al. Tumor-infiltrating lymphocytes: a predictive and prognostic biomarker in neoadjuvant treated HER2-positive breast cancer. *Clin Cancer Res.* 2016;22(23):5747–5754.
96. Solinas C, Ceppi M, Lambertini M, et al. Tumor-infiltrating lymphocytes in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy plus trastuzumab, lapatinib or their combination: a meta-analysis of randomized controlled trials. *Cancer Treat Rev.* 2017; 57:8–15.
97. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol.* 2014;25(8):1544–1550.
98. Perez EA, Thompson EA, Ballman KV, et al. Genomic analysis reveals that immune function genes are strongly linked to clinical outcome in the North Central Cancer Treatment Group n9831 Adjuvant Trastuzumab Trial. *J Clin Oncol.* 2015;33(7):701–708.
99. Zardavas D, Fouad TM, Piccart M. Optimal adjuvant treatment for patients with HER2-positive breast cancer in 2015. *Breast.* 2015; 24(suppl 2):S143–S148.

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