Neoadjuvant Breast Cancer Therapy and Drug Development

Patricia Cortazar, MD, and Paul G. Kluetz, MD

The authors are affiliated with the Office of Hematology and Oncology Products at the Center for Drug Evaluation and Research of the US Food and Drug Administration in Silver Spring, Maryland. Dr Cortazar is the clinical team leader and scientific liaison for the Breast Oncology Group in the Division of Oncology Products 1, and Dr Kluetz is the acting deputy office director.

Corresponding Author:
Patricia Cortazar, MD
Clinical Team Leader and Scientific Liaison
Breast Oncology Group
Division of Oncology Products 1 (DOP1)
Office of Hematology and Oncology Products (OHOP)
Center for Drug Evaluation and Research
US Food and Drug Administration
10903 New Hampshire Avenue
WO 22 Room 2333
Silver Spring, MD 20993
Tel: (301) 796-1346
E-mail: Patricia.Cortazar@fda.hhs.gov or pattycortazar@icloud.com

Keywords
Endocrine therapy, neodjuvant therapy, ovarian ablation, ovarian suppression, quality of life, tamoxifen

Abstract: Neoadjuvant treatment of breast cancer initially was limited to patients with locally advanced breast cancer in which downstaging was necessary. Now, neoadjuvant trials have become an increasingly common way to facilitate the rapid assessment of new cancer therapies. The appeal of neoadjuvant trials is that they provide the opportunity to study translational science, tumor biomarkers, and intermediate endpoints in response to systemic therapy within a shortened period. This review summarizes the data that contribute to our understanding of the association between pathological complete response and long-term outcomes, describes the implications of drug development and accelerated approval in neoadjuvant treatment of breast cancer, and provides a perspective on future neoadjuvant drug development.

Introduction

The use of preoperative or postoperative systemic chemotherapy as a component of combined modality treatment has been the standard of care for patients with early-stage breast cancer. It has been clearly established that there is no difference in disease-free survival (DFS) or overall survival (OS) based on the timing of chemotherapy relative to surgery.[1] Historically, the approval of new agents to treat early breast cancer has occurred up to 10 years after the initial approval in the metastatic setting. Large randomized adjuvant trials with prolonged follow-up for DFS and OS have formed the basis of early breast cancer approval. Neoadjuvant therapy has become increasingly popular, and its use has expanded beyond its initial role in tumor downstaging in order to accomplish surgery in patients with locally advanced breast cancer. The neoadjuvant setting has been appealing because it provides an ideal scenario for real-time examination of tumor tissue, imaging results, and other biomarkers in response to systemic therapy. Furthermore, it has become clear that neoadjuvant trials provide a setting in which to assess drug efficacy more expeditiously, within a

shorter time frame, and with a smaller sample size than with adjuvant trials. The increase in drug development in the neoadjuvant setting has underscored the need for a better understanding of the neoadjuvant endpoints needed to support regulatory approval.

Pathological Complete Response

Although pathological complete response (pCR) has been proposed as a surrogate endpoint for prediction of long-term clinical benefit, this surrogacy was not well established. Despite the fact that pCR was the most commonly used primary endpoint in neoadjuvant trials, it was variably defined, which made interpretation of data across clinical trials challenging. Furthermore, neoadjuvant trials typically were not powered to evaluate long-term outcomes.

To optimize the definition of pCR, enable the interpretation of data across neoadjuvant trials, and investigate the association between pCR and long-term outcome, the US Food and Drug Administration (FDA) assembled an international working group known as Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC). We conducted a pooled analysis using primary source data from nearly 12,000 patients enrolled in 12 international neoadjuvant randomized controlled trials that contained both pCR and long-term outcome data, along with a median follow-up of at least 3 years.[2]

The CTNeoBC pooled analysis had 4 main results: (1) it established pCR definitions that correlated best with long-term outcome, (2) it confirmed a better long-term outcome for individual patients who attained a pCR compared with those without a pCR, (3) it found that the prognostic value of pCR was greatest in patients with aggressive tumor subtypes, and (4) it demonstrated a weak association between long-term outcome and the magnitude of improvement in pCR rate between treatment arms.[2]

With respect to optimizing a definition for pCR, eradication of tumor from both the breast and axillary lymph nodes with or without residual carcinoma in situ (ypT0 ypN0 or ypT0/is ypN0) had a stronger association with improved event-free survival (EFS) and OS than tumor eradication from the breast alone (ypT0/is). However, the presence or absence of residual in situ carcinoma did not impact long-term outcome. By contrast, a German pooled analysis of 7 neoadjuvant trials showed that patients without residual carcinoma in situ had longer OS than did patients with residual ductal carcinoma in situ when all invasive disease was eradicated.[3] To improve consistency in future trials, the FDA proposed that pCR be defined as either ypT0 ypN0 or ypT0/is ypN0.[4] The CTNeoBC analysis also found that individual patients who attained a pCR had a 64% reduction in the risk of death compared with patients who had residual tumor at the time of surgery, supporting the prognostic value of pCR for use in clinical practice, which had been previously reported by several groups.[1,5-8]

The pooled analysis also found that the prognostic value of pCR (ypT0/is ypN0) varies according to breast cancer subtype defined by tumor grade, estrogen receptor status, and human epidermal growth factor receptor 2 (HER2) status. The highest pCR (ypT0/is ypN0) rates were found in aggressive tumor subtypes, such as triple-negative breast cancer (TNBC; 33.6%; 95% CI, 30.9%-36.4%), HER2-positive/hormone receptor–negative breast cancer treated with trastuzumab (Herceptin, Genentech; 50.3%; 95% CI, 45.0%-55.5%) or without trastuzumab (30.2%; 95% CI, 26.0%-34.5%), and grade 3 hormone receptor–positive/HER2-negative breast cancer (16.2%; 95% CI, 13.4%-19.3%). The prognostic value of pCR was also greatest in patients with aggressive tumor subtypes, in which the risk of death was reduced by 84% (95% CI, 75%-89%) in TNBC, 92% (95% CI, 78%-97%) in HER2-positive/hormone receptor–negative breast cancer treated with trastuzumab, 71% (95% CI, 50%-83%) in those who did not receive trastuzumab, and 71% (95% CI, 35%-87%) in high-grade hormone receptor–positive/HER2-negative breast cancer. As expected, there was a weak association between pCR and long-term outcome in patients with hormone receptor–positive subtypes, particularly those with low-grade breast cancer, in which pCR (ypT0/is ypN0) rates were the lowest (7.5%; 95% CI, 6.3%-8.7%).

All analyses mentioned above were patient-level analyses, also referred to as responder analyses; they compared the clinical outcome of patients with and without pCR, irrespective of the treatment arm. Although these analyses predicted improved outcome for patients who attain pCR, they are not useful for comparisons of treatments at a trial level. The CTNeoBC pooled analysis was the first large analysis in which primary source data had been used to examine the association between pCR and EFS and OS at a trial level. Surprisingly, the pooled analysis could not establish a trial-level correlation between pCR and long-term outcome. There are several potential explanations why an increase in pCR rate between treatment arms did not predict improvement in EFS and OS. Most of the trials included in the CTNeoBC pooled analysis enrolled women with heterogeneous breast cancer tumor subtypes, which could attenuate any possible association between pCR and EFS and OS. Furthermore, absolute differences in the pCR rates between treatment arms in these chemotherapy trials were low (1%-11%), except for the 20% difference in the NOAH (Neoadjuvant Herceptin) trial, which compared trastuzumab plus chemotherapy with chemotherapy alone.[9] Consistent with these results, at 5.4

years of median follow-up, patients randomly assigned to the trastuzumab arm had a 36% reduction in the risk of recurrence or death, although postoperative trastuzumab could have contributed to the improved long-term outcomes in the investigational arm.[10] The results of the NOAH trial suggest that a trial-level correlation between pCR and long-term outcome could be identified in future trials with homogeneous populations that incorporate targeted therapies achieving large absolute differences in the pCR rates between treatment arms.

Although pCR has not been validated as a surrogate endpoint for improved EFS or OS, it is considered reasonably likely that an agent that significantly improves pCR rate could predict long-term improvement in EFS or OS. This concept supports the FDA's decision to open the neoadjuvant pathway for drug approval via the accelerated approval mechanism, given the lack of a suitable endpoint short of EFS or OS to support regular approval.[4] The use of pCR as an endpoint to support accelerated approval in the neoadjuvant setting has the potential to address an unmet need in high-risk early breast cancer populations in a shorter time frame than the conventional approach to breast cancer drug development. The earlier availability of promising agents made possible by accelerated approval comes with the risk of approving an agent that ultimately may not show clinical benefit. This uncertainty can be justified for patients with early breast cancer who have a substantial risk of recurrence or death despite the best currently available therapies. To further mitigate this risk, the accelerated approval pathway will require confirmation of clinical benefit in randomized clinical trials demonstrating improved EFS, DFS, or OS.

Neoadjuvant Drug Approval

In September 2013, the FDA granted accelerated approval to pertuzumab (Perjeta, Genentech) as a component of neoadjuvant treatment for locally advanced, inflammatory, or early-stage HER2-positive breast cancer.[11] The approval was based on NeoSphere (A Study of Pertuzumab in Combination With Herceptin in Patients With HER2 Positive Breast Cancer), a randomized trial in 417 patients with HER2-positive, operable, locally advanced, or inflammatory breast cancer that demonstrated a statistically significant 18% absolute improvement in pCR rate with the addition of pertuzumab to docetaxel and trastuzumab.[9] Given the lack of experience with pCR as a regulatory endpoint and uncertainty about the association between pCR rate and long-term outcome, the bar for approval in the neoadjuvant setting was set high. The pertuzumab approval was evaluated within the context of the totality of the efficacy and safety data for the drug. Critical to the decision for the neoadjuvant approval of pertuzumab was its comprehensive clinical development program, particularly the substantial improvement in OS in first-line, metastatic, HER2-positive breast cancer with the addition of pertuzumab to a backbone of docetaxel and trastuzumab demonstrated in the phase 3 CLEOPATRA (A Study to Evaluate Pertuzumab + Trastuzumab + Docetaxel vs. Placebo + Trastuzumab + Docetaxel in Previously Untreated HER2-Positive Metastatic Breast Cancer) trial.[12,13] Additionally, at the time of accelerated approval the adjuvant confirmatory trial (APHINITY; Adjuvant Pertuzumab and Herceptin in Initial Therapy of Breast Cancer), comparing standard chemotherapy and trastuzumab plus 1 year of pertuzumab vs chemotherapy and trastuzumab alone, completed accrual of more than 4800 patients, with the first DFS results anticipated in late 2016. This robust development program, with well-characterized efficacy and safety in approximately 10,000 patients who had received pertuzumab, mitigated the potential risks of accelerated approval utilizing increased pCR rate.

The strength of pCR as an efficacy endpoint will continue to be assessed as more data become available. It is possible that randomized trials of targeted agents in homogeneous tumor subtypes with larger differences in pCR rates will demonstrate a relationship between pCR and long-term outcome at the trial level. However, a recent discrepancy between the pCR improvement achieved with the addition of lapatinib (Tykerb, GlaxoSmithKline) to neoadjuvant paclitaxel and trastuzumab (51.3%; 95% CI, 43.1%-59.5% vs 29.5%; 95% CI, 22.4%-37.5%) in the NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation) trial and the long-term outcome with the same dual anti-HER2 adjuvant therapy in the ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation) trial were reported.[14,15] With 4.5 years of median follow-up, DFS was not significantly improved with the combination of lapatinib and trastuzumab compared with trastuzumab (hazard ratio, 0.84; 97.5% CI, 0.70-1.02; P=.048 with $P \leq .025$ required for statistical significance). The lower-than-anticipated number of events at the time of the DFS analysis in the patient population with a lower risk of recurrence could in part account for the trial failing to meet its primary endpoint. Though the results are disappointing, they are not surprising in view of the modest contribution of lapatinib to the efficacy of the dual anti-HER2 therapy in metastatic breast cancer. Moreover, it is not clear why the results from NeoALTTO also failed to predict the eventual inferiority of the lapatinib arm in the ALTTO trial.[14]

Although several studies have confirmed an increase in the pCR rate with the addition of lapatinib and trastuzumab compared with trastuzumab alone, there is a wide range in the magnitude of pCR improvement with this dual HER2 regimen across neoadjuvant trials.[16-20]

Though the magnitude of pCR improvement needed to improve long-term outcomes remains unclear, only the CHER-LOB (Chemotherapy, Herceptin and Lapatinib in Operable Breast Cancer) study replicated the magnitude of improvement seen in the NeoALTTO study, and 3 studies (NSABP-41, CALGB 40601, and LAPATAX) showed a small increase in pCR after neoadjuvant dual HER2 blockade with lapatinib and trastuzumab.[16-19] The differences in pCR among the various studies can be in part attributed to the different chemotherapy backbone used in each study, including the variable durations of preoperative anti-HER2 therapy. The CTNeoBC group is in the process of conducting analyses of additional anti-HER2 trials that will enable the interpretation of data to investigate the relationship between pCR and long-term outcome at a trial level. In the meantime, the FDA will continue to evaluate pCR as a potential surrogate endpoint in populations known to remain at high risk for disease recurrence and death, particularly when supportive data exist in the metastatic setting, as was seen with the pertuzumab program.

Though patients who attain pCR regardless of treatment arm had a better survival outcome, it is important to emphasize the challenges associated with the interpretation of pCR in neoadjuvant trials. For example, 14% of patients who achieved pCR in the CTNeoBC pooled analysis had a subsequent recurrence or death within 5 years, whereas a substantial number of patients with residual disease remained event-free.[2] Despite the low pCR rates in patients with low-grade, hormone receptor–positive tumors, these patients have a favorable long-term prognosis and are more likely to be cured with currently available therapy, rendering pCR a poor predictor of clinical benefit in this population. Furthermore, patients with hormone receptor–positive breast cancer typically receive postoperative endocrine therapy, which contributes to the favorable long-term outcome. Unfortunately, there is currently no way to distinguish patients who can be cured with existing treatments from patients who will have disease relapse. Consequently, intensive efforts are being devoted to identify novel molecular biomarkers that can better identify high-risk patients and predict tumor response to neoadjuvant therapy in order to facilitate the development of treatments that can improve long-term clinical benefit.

Biomarkers

The recent increase in biomarker-targeted drug development underscores the need for independent validation studies to establish novel prognostic and predictive biomarkers. Progress in predictive markers and further studies on the molecular background of patients with a poor prognosis can facilitate the development of new effective therapies for patients resistant to neoadjuvant therapies. Likewise, prognostic biomarkers also could identify patients with good prognostic signatures for whom aggressive therapies would not be necessary. Estrogen receptor (ER) and HER2 status are the only well-established prognostic and predictive biomarkers in breast cancer.[2,21-24] Therefore, there is a great need to validate additional predictive and prognostic biomarkers in better defined breast cancer subtypes to help establish drug efficacy and expedite development of highly effective agents for patients with high-risk early breast cancer.

Newer techniques for assessing residual disease burden have been studied. The residual cancer burden (RCB) index and the clinical-pathologic stage + ER status/tumor grade (CPS + EG) scoring system are prognostic tools that incorporate response to therapy in addition to other clinical and pathological factors. The RCB index describes a spectrum of residual disease determined in the tumor bed and lymph nodes, and the amount of residual disease present has been shown to correlate with outcome.[25,26] The potential benefit of RCB over pCR is the ability to identify patients with minimal residual disease (RCB-I) for whom prognosis and long-term outcomes are similar to those who attain a pCR (RCB-0). The CPS + EG scoring system uses clinical stage before treatment, pathological stage after treatment, ER status, and tumor grade. Based on the scoring system, patients are divided into 7 groups (scores 0-6) with different 5-year long-term outcomes.[27]

Neoadjuvant chemotherapy or endocrine therapy in ER-positive breast cancer has been associated with a slower response and a lower pCR rate, supporting the need for alternative endpoints. Ki67 is an independent prognostic proliferation marker and its suppression has been observed following neoadjuvant endocrine or cytotoxic therapies.[28-31] Von Minckwitz reported that patients with a Ki67 level of greater than 35% after neoadjuvant chemotherapy had a worse outcome than those with levels of 35% or less.[28] Additionally, the suppression of Ki67 during neoadjuvant endocrine therapy predicted recurrence-free survival in adjuvant endocrine trials with matched treatment randomizations. [29,32,33] Despite several studies indicating that Ki67 suppression in the neoadjuvant setting could potentially be used to predict outcome, Ki67 lacks scoring standardization and prospective validation has not been demonstrated.[34]

The preoperative endocrine prognostic index (PEPI) model developed by Ellis and colleagues is another attempt to understand the relationship between tumor response and risk of relapse. The PEPI score integrates information on pathological staging after neoadjuvant endocrine therapy with ER status and levels of Ki67 in the surgical specimen.[35] This model was studied in a cohort of patients from the IMPACT neoadjuvant endocrine

trial.[36] The authors concluded that patients with pathological stage I or 0 tumors after neoadjuvant endocrine therapy and a PEPI score of 0 had a low risk of relapse and were unlikely to benefit from adjuvant chemotherapy. The ongoing ALTERNATE trial will prospectively evaluate the modified PEPI score in postmenopausal women treated with neoadjuvant fulvestrant, anastrozole, or the combination of both hormonal therapies. The PEPI tool is not currently validated and the prognostic information only becomes available after treatment. Earlier markers of response to identify tumors that are not responding to neoadjuvant endocrine therapy are needed.

It is important to consider that breast cancer is molecularly heterogeneous. There are 4 intrinsic molecular subtypes of breast cancer that have been identified using gene expression profiling: luminal A, luminal B, HER2-enriched (HER2E), and basal-like.[37,38] Several trials have evaluated multigene assays as predictors of response to therapy in the neoadjuvant setting, and validation efforts are ongoing.[39,40] Intrinsic subtypes appear to differ in sensitivity to HER2-targeting agents, with numerically highest pCR rates among the HER2E subtype.[41] Subset analyses from the CALGB (Cancer and Leukemia Group B) 40601 and the NOAH trials, using PAM50 to identify the intrinsic subtypes, indicated the HER2E subgroup had the greatest pCR rates when treated with anti–HER2-based chemotherapy, compared with the other subtypes.[19,42] Recent gene expression profiling has identified up to 6 distinct TNBC subtypes (2 basal-like, 1 immunomodulatory, 1 mesenchymal, 1 mesenchymal stem-like, and 1 luminal androgen receptor subtype).[43,44] In the CALGB 40603 neoadjuvant trial, a PAM50 analysis performed on pretreatment TNBC samples showed that the basal-like gene expression profile predicted a greater pCR increment with the addition of bevacizumab to carboplatin.[45,46]

The *BRCA1* and *BRCA2* genes, which are critical in the homologous recombination DNA repair pathway, have been studied as predictors of response in neoadjuvant trials. Tumors with *BRCA1* and *BRCA2* mutations have decreased DNA repair and are highly sensitive to DNA-damaging agents. Three studies have suggested the association of *BRCA1* mutations with improved pCR rates.[47-49] Specific interest also has been related to the use of platinum agents in patients with *BRCA* mutations. Byrski and coinvestigators recently reported the results of a prospective study with single-agent cisplatin that showed a high pCR rate of 61% in *BRCA1*-mutated patients.[50] The GeparSixto (Addition of Carboplatin to Neoadjuvant Therapy for Triple-negative and HER2-Positive Early Breast Cancer) study also demonstrated a higher pCR rate after neoadjuvant anthracycline/taxane/carboplatin-based chemotherapy in TNBC patients who are *BRCA* mutation carriers.[51] Additionally, the GeparSixto and PrECOG 0105 (Phase II Study of Gemcitabine, Carboplatin, and Iniparib as Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation-Associated Breast Cancer With Assessment of a Tumor-Based Measure of Genomic Instability) studies reported higher pCR rates with platinum-based regimens in patients with elevated homologous recombination deficiency (HRD) scores. Further confirmation of the effect of neoadjuvant treatment regimens in *BRCA* mutation carriers or in the homologous recombination pathway are needed to establish the association of HRD scores with response to neoadjuvant chemotherapy in patients with TNBC.[52,53]

Specific pathways activated in breast cancer have been examined in the neoadjuvant setting as potential biomarkers. The activation of the phosphoinositide 3-kinase (PI3K) pathway has been associated with HER2-targeted therapy resistance and with higher pCR.[54] The German Breast Group examined *PIK3CA* mutations in 3 large HER2-positive neoadjuvant studies (GeparQuattro, GeparQuinto, and GeparSixto) and reported that patients harboring a *PIK3CA* mutation achieved a lower pCR rate (19.4%) compared with those with *PIK3CA* wild-type tumors (32.8%).[55] Though confirmation of these results was observed in a combined analysis from almost 1000 patients from the German Breast Group, NeoALTTO, and CHER-LOB studies, there was no difference in long-term outcome between the *PIK3CA*-mutant and *PIK3CA*–wild-type cohorts.[56]

Considering one of the main challenges in drug development of targeted therapies is the molecular heterogeneity of the tumors, understanding the role of the immune system in eradicating or controlling cancer may hold promise. Additionally, the unprecedented efficacy recently reported with immune checkpoint inhibitors in the treatment of melanoma and lung cancer has encouraged studies of tumor immunology in breast cancer. Recent studies have found that the presence of tumor-infiltrating lymphocytes (TILs) in tumor samples predicts response to anthracycline/taxane-based neoadjuvant therapy.[57,58] In one study, the presence of TILs in the core biopsies prior to neoadjuvant HER2-directed therapy was associated with a higher pCR rate.[59] Although several studies support TILs as a prognostic marker in TNBC,[60,61] the prognostic significance of TILs in HER2-positive disease is controversial. A recent report from the NeoALTTO trial in HER2-positive breast cancer supported the association of high levels of stromal TILs with improved pCR and EFS after neoadjuvant therapy. Likewise, the FinHER (Finland Herceptin) trial also suggested a relationship between a high level of TILs and long-term outcome after adjuvant chemotherapy.[59,62] In contrast, a large analysis from the adjuvant Alliance N9831 trial did not associate

a high level of TILs with trastuzumab benefit, but it was predictive of chemotherapy benefit.[63] Further validation of level of TILs will be needed before it can be reliably used as a prognostic and/or predictive biomarker.

A key immune modulatory pathway is mediated by the axis between programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1). The expression of PD-L1 in tumor cells or in the tumor microenvironment has been correlated with the presence of TILs. Wimberly and colleagues recently reported that both PD-L1 and TILs correlated with pCR, and high PD-L1 predicted pCR in a multivariate analysis.[64] Additionally, in a pooled series, upregulated PD-L1 expression was correlated with pCR in basal and HER2-enriched cases, and upregulated cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) was shown to demonstrate improved outcomes after trastuzumab therapy.[62,65]

Conclusions

Recent findings from genomic studies suggest that breast cancer is a group of diseases characterized by distinct genomic abnormalities that have significant differences in prevalence, risk factors, prognosis, and response to treatment. Conventional drug development should be adapted to utilize biomarkers that identify populations who are at high risk for relapse and predict those who will derive the most benefit in order to develop effective therapies for the patients with greatest unmet need. Neoadjuvant trials present an opportunity to introduce innovative approaches to improve the drug development paradigm. Pathological response to preoperative therapy reflects the complex interaction of tumor biology, tumor microenvironment, and systemic therapy. Future neoadjuvant trials conducted in more narrowly defined breast cancer subtypes using validated biomarkers could result in larger improvements in pCR rates and shed light on the value of pCR in breast cancer drug development. Further development and use of biomarkers in neoadjuvant breast cancer trials can help to expedite the availability of highly effective agents to the patients in greatest need.

Disclosures

The authors have no conflicts of interest.

References

1. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr.* 2001(30):96-102.
2. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet*. 2014;384(9938):164-172.
3. Cortazar P, Geyer CE Jr, Gianni L, Cameron D, von Minckwitz G. Pathological complete response in breast cancer—authors' reply. *Lancet*. 2015;385(9963):114-115.
4. U.S. Food and Drug Administration. Guidance for industry: pathological complete response in neoadjuvant treatment of high-risk early-stage breast cancer: use as an endpoint to support accelerated approval. Published October 2014.
5. von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*. 2012;30(15):1796-1804.
6. Bear HD, Anderson S, Brown A, et al; National Surgical Adjuvant Breast and Bowel Project Protocol B-27. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol*. 2003;21(22):4165-4174.
7. von Minckwitz G, Untch M, Nüesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. *Breast Cancer Res Treat*. 2011;125(1):145-156.
8. Feldman LD, Hortobagyi GN, Buzdar AU, Ames FC, Blumenschein GR. Pathological assessment of response to induction chemotherapy in breast cancer. *Cancer Res*. 1986;46(5):2578-2581.
9. Gianni L, Pienkowski T, Im YH, 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.
10. 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.
11. Amiri-Kordestani L, Wedam S, Zhang L, et al. First FDA approval of neoadjuvant therapy for breast cancer: pertuzumab for the treatment of patients with HER2-positive breast cancer. *Clin Cancer Res*. 2014;20(21):5359-5364.
12. Baselga J, Cortés J, Kim SB, et al; CLEOPATRA Study Group. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366(2):109-119.
13. Swain SM, Kim SB, Cortés J, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA study): overall survival results from a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Oncol*. 2013;14(6):461-471.
14. Baselga J, Bradbury I, Eidtmann H, et al; NeoALTTO Study Team. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2012;379(9816):633-640.
15. Piccart-Gebhart MJ, Holmes AP, Baselga J, et al. First results from the phase III ALTTO trial (BIG 2-06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone, trastuzumab alone, their sequence, or their combination in the adjuvant treatment of HER2-positive early breast cancer [ASCO abstract LBA4]. *J Clin Oncol*. 2014;32(5)(suppl).
16. Robidoux A, Tang G, Rastogi P, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2013;14(12):1183-1192.
17. Guarneri V, Frassoldati A, Bottini A, et al. Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: results of the randomized phase II CHER-LOB study. *J Clin Oncol*. 2012;30(16):1989-1995.
18. Bonnefoi H, Jacot W, Saghatchian M, et al. Neoadjuvant treatment with docetaxel plus lapatinib, trastuzumab, or both followed by an anthracycline-based chemotherapy in HER2-positive breast cancer: results of the randomised phase II EORTC 10054 study. *Ann Oncol*. 2015;26(2):325-332.
19. Carey LA, Berry DA, Ollila D, et al. Clinical and translational results of CALGB 40601: a neoadjuvant phase III trial of weekly paclitaxel and trastuzumab with or without lapatinib for HER2-positive breast cancer [ASCO abstract 500]. *J Clin Oncol*. 2013;31(15)(suppl).
20. Holmes FA NY, Espina VA et al Correlation of molecular effects and pathologic complete response to preoperative lapatinib and trastuzumab, separately and combined prior to neoadjuvant breast cancer chemotherapy [ASCO abstract 506]. *J Clin Oncol*. 2011;29(7)(suppl).
21. Ring AE, Smith IE, Ashley S, Fulford LG, Lakhani SR. Oestrogen receptor status, pathological complete response and prognosis in patients receiving neoadjuvant chemotherapy for early breast cancer. *Br J Cancer*. 2004;91(12):2012-2017.
22. Colleoni M, Viale G, Zahrieh D, et al. Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. *Clin Cancer Res*. 2004;10(19):6622-6628.
23. Keam B, Im SA, Kim HJ, et al. Prognostic impact of clinicopathologic parameters in stage II/III breast cancer treated with neoadjuvant docetaxel and doxorubicin chemotherapy: paradoxical features of the triple negative breast cancer. *BMC Cancer*. 2007;7:203.

24. Petit T, Wilt M, Velten M, et al. Semi-quantitative evaluation of estrogen receptor expression is a strong predictive factor of pathological complete response after anthracycline-based neo-adjuvant chemotherapy in hormonal-sensitive breast cancer. *Breast Cancer Res Treat*. 2010;124(2):387-391.
25. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. *J Clin Oncol*. 2007;25(28):4414-4422.
26. Aguirre Ortega EM, Taberner Bonastre M, Morales Murillo S, et al. Primary systemic chemotherapy in stage II-IIIb breast cancer: independent prognostic value of molecular phenotype and the MDACC residual cancer burden—a single-institutional experience. [ASCO abstract e21132]. *J Clin Oncol.* 2011;29(7)(suppl).
27. Mittendorf EA, Jeruss JS, Tucker SL, et al. Validation of a novel staging system for disease-specific survival in patients with breast cancer treated with neoadjuvant chemotherapy. *J Clin Oncol*. 2011;29(15):1956-1962.
28. von Minckwitz G, Schmitt WD, Loibl S, et al. Ki67 measured after neoadjuvant chemotherapy for primary breast cancer. *Clin Cancer Res*. 2013;19(16):4521-4531.
29. Dowsett M, Smith IE, Ebbs SR, et al; IMPACT Trialists Group. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst*. 2007;99(2):167-170.
30. Dowsett M, Smith IE, Ebbs SR, et al; IMPACT Trialists. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. *Clin Cancer Res*. 2005;11(2 pt 2):951s-958s.
31. Jones RL, Salter J, A'Hern R, et al. The prognostic significance of Ki67 before and after neoadjuvant chemotherapy in breast cancer. *Breast Cancer Res Treat*. 2009;116(1):53-68.
32. Ellis MJ, Ma C. Letrozole in the neoadjuvant setting: the P024 trial. *Breast Cancer Res Treat*. 2007;105(suppl 1):33-43.
33. Howell A, Cuzick J, Baum M, et al; ATAC Trialists' Group. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. 2005;365(9453):60-62.
34. Harris L, Fritsche H, Mennel R, et al; American Society of Clinical Oncology. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007;25(33):5287-5312.
35. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst*. 2008;100(19):1380-1388.
36. Smith IE, Dowsett M, Ebbs SR, et al; IMPACT Trialists Group. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol*. 2005;23(22):5108-5116.
37. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
38. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70.
39. Ayers M, Symmans WF, Stec J, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *J Clin Oncol.* 2004;22(12):2284-2293.
40. Gianni L, Zambetti M, Clark K, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol*. 2005;23(29):7265-7277.
41. Prat A, Carey LA, Adamo B, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. *J Natl Cancer Inst*. 2014;106(8):dju152.
42. Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clin Cancer Res*. 2014;20(2):511-521.
43. Perou CM. Molecular stratification of triple-negative breast cancers. *Oncologist*. 2011;16(suppl 1):61-70.
44. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750-2767.
45. Sikov WM, Barry WT, Hoadley KA, et al. Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT) +/- carboplatin (Cb) or bevacizumab (Bev): CALGB 40603/150709 (Alliance) [2014 SABCS abstract S4-05]. *Cancer Res*. 2015;75(9)(suppl).
46. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol*. 2015;33(1):13-21.
47. Byrski T, Huzarski T, Dent R, et al. Pathologic complete response to neoadjuvant cisplatin in BRCA1-positive breast cancer patients. *Breast Cancer Res Treat*. 2014;147(2):401-405.
48. Wang C, Zhang J, Wang Y, et al. Prevalence of BRCA1 mutations and responses to neoadjuvant chemotherapy among BRCA1 carriers and non-carriers with triple-negative breast cancer. *Ann Oncol*. 2015;26(3):523-528.
49. Telli ML, Jensen KC, Kurian AW, et al. PrECOG 0105: final efficacy results from a phase II study of gemcitabine (G) and carboplatin (C) plus iniparib (BSI-201) as neoadjuvant therapy for triple-negative (TN) and BRCA1/2 mutation-associated breast cancer [ASCO abstract 1003]. *J Clin Oncol*. 2013;31(15)(suppl).
50. Arun B, Bayraktar S, Liu DD, et al. Response to neoadjuvant systemic therapy for breast cancer in BRCA mutation carriers and noncarriers: a single-institution experience. *J Clin Oncol*. 2011;29(28):3739-3746.
51. Von Minckwitz G, Hahnen E, Fasching PA, et al. Pathological complete response (pCR) rates after carboplatin-containing neoadjuvant chemotherapy in patients with germline BRCA (gBRCA) mutation and triple-negative breast cancer (TNBC): Results from GeparSixto [ASCO abstract 1005].
52. Von Minckwitz G, Timms K, Untch M, et al. Prediction of pathological complete response (pCR) by Homologous Recombination Deficiency (HRD) after carboplatin-containing neoadjuvant chemotherapy in patients with TNBC: results from GeparSixto [ASCO abstract 1004]. *J Clin Oncol.* 2015;33(15)(suppl).
53. Telli ML, Jensen KC, Vinayak S, et al. Phase II study of gemcitabine, carboplatin, and iniparib as neoadjuvant therapy for triple-negative and BRCA1/2 mutation-associated breast cancer with assessment of a tumor-based measure of genomic instability: PrECOG 0105. *J Clin Oncol*. 2015;33(17):1895-1901.
54. Dave B, Migliaccio I, Gutierrez MC, et al. Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. *J Clin Oncol*. 2011;29(2):166-173.
55. Loibl S, von Minckwitz G, Schneeweiss A, et al. PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (HER2) therapy in primary HER2-overexpressing breast cancer. *J Clin Oncol*. 2014;32(29):3212-3220.
56. Loibl S, Majewski I, Guarneri V, et al. Correlation of PIK3CA mutation with pathological complete response in primary HER2-positive breast cancer: combined analysis of 967 patients from three prospective clinical trials [ASCO abstract 511]. *J Clin Oncol.* 2015;33(15)(suppl).
57. Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol*. 2010;28(1):105-113.
58. West NR, Milne K, Truong PT, Macpherson N, Nelson BH, Watson PH. Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer. *Breast Cancer Res*. 2011;13(6):R126.
59. Salgado R, Denkert C, Campbell C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. *JAMA Oncol.* 2015;1(4):448-454.
60. Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol*. 2014;32(27):2959-2966.
61. Dieci MV, Criscitiello C, Goubar A, et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. *Ann Oncol.* 2014;25(3):611-618.
62. 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.
63. Perez EA, Ballman KV, Anderson SK. Stromal tumor-infiltrating lymphocytes (S-TILs): in the Alliance N9831 trial S-TILSs are associated with chemotherapy benefit but not associated with trastuzumab benefit [2014 SABCS abstract S1-06]. *Cancer Res*. 2015;75(9)(suppl).
64. Wimberly H, Brown JR, Schalper K, et al. PD-L1 Expression correlates with tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy in breast cancer. *Cancer Immunol Res*. 2015;3(4):326-332.
65. Sabatier R, Finetti P, Mamessier E, et al. Prognostic and predictive value of PDL1 expression in breast cancer. *Oncotarget*. 2015;6(7):5449-5464.