TBCRC023: A Randomized Phase II Neoadjuvant Trial of Lapatinib Plus Trastuzumab Without Chemotherapy for 12 vs. 24 Weeks in Patients with HER2-positive Breast Cancer

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Translational Relevance:

Neoadjuvant lapatinib plus trastuzumab without chemotherapy, has been previously reported to yield pathologic complete responses in a subset of patients with HER2-positive breast cancer. The optimal duration of anti-HER2 therapy in the context of a no chemotherapy regimen, however, remains unclear. In this study, longer duration of neoadjuvant therapy (24 weeks) with lapatinib plus trastuzumab achieved higher pCR rates compared to 12 weeks, particularly in patients with HER2-positive/ER-positive disease. Our findings, if validated in a larger trial, argue that the optimal duration of therapy for different subgroups (ER-positive vs. ER-negative) in HER2-positive breast cancer may be different. Our results further support the need to accurately identify patients based on the clinical and biologic characteristics of each tumor and each patient for tailored treatments, and for appropriate therapy escalation or de-escalation.

Abstract:

Purpose: Prior neoadjuvant trials with 12 weeks of dual anti-HER2 therapy without chemotherapy demonstrated a meaningful pathologic complete response (pCR) in patients with HER2-positive breast cancer. In this trial, we sought to determine whether longer treatment would increase the rate of pCR.

Methods: TBCRC023 (NCT00999804) is a randomized phase II trial combining a Simon Phase 2 design in the experimental arm with a pick-the-winner design, not powered for direct comparison. Women with HER2-positive breast tumors measuring ≥2 cm (median=5 cm) were randomized in a 1:2 ratio to 12 vs. 24 weeks of lapatinib and trastuzumab. Letrozole (along with ovarian suppression if premenopausal) was administered in patients whose tumors were also ER-positive. All evaluable patients were assessed for in-breast pCR.

Results: Ninety-seven patients were enrolled (33 in 12-wk arm and 64 in 24-wk arm), of whom 94 were evaluable. Median age was 51 and 55% were postmenopausal. Median tumor size was 5 cm and 65% were ER-positive. The rate of pCR in the 24-wk arm was 28% and numerically superior to the 12-week arm (12%). This was driven by increased pCR in the ER-positive subgroup (33% vs. 9%). Study treatment was well tolerated with grade 1-2 diarrhea and acneiform rash being the most common toxicities.

Conclusions: Treatment with dual anti-HER2 therapy for 24 weeks led to a numeric increase in pCR rate in women with HER2-positive breast cancer, without using chemotherapy. If validated, this approach may help identify patients who may benefit from de-escalation of therapy.

Introduction

Breast tumors with overexpression or amplification of the human epidermal growth factor receptor 2 gene (*ERBB2* or *HER2*) comprise 15-20% of breast cancer (1). These HER2-positive breast cancers have an aggressive clinical behavior, and used to be associated with poor prognosis. However, the introduction of the monoclonal anti-HER2 antibody trastuzumab has dramatically improved outcomes when added to combination chemotherapy (2-4).

HER2 belongs to a membrane tyrosine kinase family (HER1-4). This family works in a coordinated fashion to activate downstream signaling pathways which regulate cell proliferation, survival, angiogenesis, differentiation, invasion and metastasis (5). Using two different combinations of anti-HER agents, trastuzumab together with either lapatinib or pertuzumab, our preclinical data were the first to demonstrate that HER2-positive xenograft tumors could be eradicated in an *in vivo* model (6-8). However, with both combinations of anti-HER2 agents, simultaneous blockade of ER was required to enhance efficacy.

Results of several independent clinical trials validated the concept that potent inhibition of HER2 with combination therapy targeting HER receptors (commonly referred to as dual anti-HER2 therapy) without the use of chemotherapy yields a clinically meaningful rate of pathologic complete response (pCR) in the neoadjuvant setting (9-11). The NeoSphere trial showed a pCR rate of 17% in the arm with trastuzumab plus pertuzumab and no chemotherapy (11). In the TBCRC006 trial (NCT00548184), we showed that the combination of lapatinib and trastuzumab (with the addition of endocrine therapy in patients with ER-positive tumors) for 12 weeks resulted in a pCR rate of 27% in patients with a median tumor size of 6 cm (9). The PAMELA

trial reported by Prat and colleagues, using the same dual anti-HER2 regimen administered for 18 weeks, reported an overall pCR rate of 30% (10).

These studies suggest that a subgroup of patients may not require chemotherapy, thus sparing these patients the unnecessary toxicity and cost of that treatment. Other studies in the neoadjuvant setting that combined chemotherapy with dual HER2 inhibition demonstrated increased rates of pCR with more complete blockade of the HER family, though in some cases it was modest and not statistically significant (11-14).

An intriguing finding from TBCRC006 was that 22% of patients had residual invasive disease of ≤ 1cm (ypT_{1a-b}) despite a median baseline tumor size of 6 cm. Most of these responses were observed in ER-positive tumors (9). This raised the questions of optimal treatment duration and whether a longer duration beyond 12 weeks may result in higher pCR rates, especially in ER-positive tumors. If so, this may help increase the proportion of patients achieving pCR who may not require chemotherapy.

We therefore hypothesized that neoadjuvant treatment for 24 weeks with dual anti-HER2 therapy without chemotherapy, plus endocrine therapy for ER-positive disease, would increase pCR rates in patients with HER2 positive breast cancer over the same treatment administered for only 12 weeks.

Patients and Methods

TBCRC023 (NCT00999804) was a randomized multicenter phase II study conducted in collaboration with the Translational Breast Cancer Research Consortium (**Figure 1A**).

Institutional review board and scientific committee approval were obtained at the lead site (Dan L Duncan Comprehensive Cancer Center at Baylor College of Medicine) and other participating sites. Written informed consent was obtained from all patients.

Eligible patients were 18 years of age or older with histologically confirmed invasive breast carcinomas that were HER2-positive, assessed by immunohistochemistry (IHC) or fluorescence *in situ* hybridization, assessed locally according to the 2007 ASCO/CAP guidelines (15). Breast tumors needed to be 2 cm or larger by clinical measurement, with any nodal status. Good performance status (ECOG performance status 0-2) and adequate organ function were also required.

Study participants were randomized in a ratio of 1:2 to receive 12 vs. 24 weeks of lapatinib (Tykerb, supplied by GlaxoSmithKline [London, UK]) 1000 mg orally every day and trastuzumab 4 mg/kg loading dose followed by 2 mg/kg per week. If the tumor was ER-positive and/or progesterone receptor (PR)-positive by IHC (according to the 2010 ASCO/CAP guidelines) (16), patients were also treated with letrozole 2.5 mg orally once per day (combined with LHRH agonist of physician's choice in premenopausal women).

Tumor tissue was collected at baseline (before treatment), and after 1 week and at the time of surgery (or completion of study treatment). Tumor biopsies were also performed at 12 weeks on patients on the 24-week arm, if they had residual tumor by ultrasound per the judgement of the local radiologist. Collected samples were divided with a portion placed in formalin for subsequent paraffin embedding [formalin-fixed paraffin embedded tissue (FFPE)] and a portion flash frozen on dry ice.

Upon completion of study treatment, patients proceeded to surgery. However, further neoadjuvant treatment with chemotherapy was allowed, if deemed clinically necessary. Adjuvant therapy after surgery was at the discretion of the treating physician.

The study measured the rate of pCR (disappearance of all invasive tumor in the breast, $ypT_{0\text{-}is}$) at the time of surgery. Pathologic assessment of response was assessed according to the local institutional guidelines and practice. Patients who did not proceed to surgery, or received additional neoadjuvant therapy prior to surgery, were considered as not achieving the primary endpoint of pCR.

In this trial, while the protocol mandated the collection of only grade 3 and 4 toxicity data, lower grade toxicities reported by study participants were also recorded as part of the routine on-study assessment. Protocol-defined reportable adverse events consisted of toxicity of any grade that required a modification to treatment (*e.g.*, dose modification, dose delay, or drug discontinuation); all liver toxicity regardless of grade; all ≥ grade 2 cardiac or pulmonary toxicity; abnormal laboratory values or diagnostic test results (if they induced clinical signs or symptoms or required treatment or further diagnostic tests); all grade 3 and 4 adverse events, regardless of causality; and all serious adverse events (SAEs).

The design of this randomized trial combined an admissible Simon-like Phase 2 design (17), which minimizes both the number of subjects enrolled on an ineffective treatment and minimizes the total sample size, for the extended (24 weeks) arm, AND, a pick-the-winner-like requirement, at the conclusion of the trial, of numeric superiority of 24 week pCR rate over 12

week pCR rate. Randomization (2:1 in favor of 24 weeks) was stratified by ER status (ER-positive, ER-negative). The trial was designed to detect an increase in the rate of pCR in the 24-week arm from 27% observed at first report of TBCRC006 to 45%, which required up to 55 evaluable patients. The first stage of 31 patients (successful interim analysis conducted November, 2013) required 10 or more responses in order to proceed for an additional 24 evaluable patients (total of 55). At the end of the trial, if 20 or more subjects responded, AND the pCR rate with extended therapy was numerically superior to the control (12 week) pCR rate, we could conclude in favor of extended therapy. Based on simulations, we had 85% power overall to detect a true response rate of 45% in the extended arm AND numeric superiority over control (assume control pCR rate=28%), and no more than a 10% type I error when the extended arm pCR rate was 28% or less. The 12-week control arm did not have an early stopping rule. It accrued while the 24-week arm was open, with maximum enrollment of 28 evaluable subjects (half of the 24-week arm sample size). The control arm allowed an internal benchmark for the overall trial without the need to rely on historical data, including our own TBCRC 006 study that also tested a 12-week duration of therapy.

On April 12, 2013 a protocol amendment was approved to allow the enrollment of an expansion cohort of 40 participants, as long as the trial did not meet other stopping rules, to better characterize the correlative science objectives of the study. No change was made to the trial's primary endpoint or to the efficacy assumptions, and we report here the outcome data based on the pre-specified efficacy cohort before the addition of the expansion cohort.

Results

Between November 2011 and November 2013, eligible patients were enrolled at eight sites (Baylor College of Medicine Dan L Duncan Comprehensive Cancer Center, Vanderbilt University, University of Alabama in Birmingham, Johns Hopkins Sidney Kimmel Cancer Center, University of Chicago, Indiana University, Dana Farber Cancer Institute, and Mayo Clinic).

Of the 100 patients screened, ninety-seven patients were enrolled and randomized in a 1:2 ratio to the 12 vs. the 24-week arm (**Figure 1B**, CONSORT diagram). Thirty-one out of the 33 patients randomized to the 12-week arm completed study treatment. Two patients discontinued therapy early on this arm, one due to progressive disease, and one due to toxicity (**Supplementary Table 1)**. The study treatment was otherwise well tolerated with grade 1-2 diarrhea and acneiform rash being the most common toxicities. Tumor biopsies were collected at baseline (before treatment), and at different time points thereafter, as described in the methods, from evaluable randomized patients (**Table 1**).

Three patients in the 24-week arm were found ineligible after they received treatment: two had metastatic disease at baseline, and 1 had disease that was FISH negative and was taken off the study by her doctor before completing one cycle of lapatinib. Forty-seven out of 61 eligible patients on the 24-week arm completed study treatment. Fourteen patients discontinued treatment early (**Supplementary Table 1**). Seven were removed due to disease progression, three due to toxicity, and four withdrew consent; all of these were considered as not achieving pCR in the intent-to-treat analysis. The characteristics of patients with disease progression in the 12-week and 24-week arms are summarized in **Supplementary Table 2**.

Table 2 summarizes patient characteristics. Median age was 51, and 45% of the patients were premenopausal. Study participants came from diverse racial and ethnic backgrounds (listed in the table). Study participants had tumors with high-risk features, including a median tumor size of 5 cm (range 0-15 cm), nearly a third with stage III disease, and 71% with histologic grade 3. Almost two thirds of patients (65%) had tumors that were ER positive.

The overall pCR rate in the breast was lower than expected. The 24-week arm passed Stage 1 of the study design but in Stage 2, did not meet the requirement for at least 20 pCRs out of the first 55 response-evaluable participants. Overall, the pCR rate was 12% in the 12-week arm and 28% in the 24-week arm (**Table 3**). This increase was seen entirely in the ER-positive subgroup, where the pCR was 9% in the 12-week arm and 33% in the 24-week arm. The pCR rate in the ER-negative subgroup was similar in both study arms, with 20% in the 12-week arm and 18% in the 24-week arm.

Figure 2 shows pathologic responses in each treatment arm by ER status. In ER positive tumors, 30% of the patients in the 12-week arm had residual tumors of 1 cm or less, while only 13% of patients in the 24-week arm had that response. In the ER-negative group, there were no patients with residual disease of 1 cm or less in the 12-week arm, while 18% of patients in the 24-week arm had that response. A total of 8 patients (8.5%) were taken off study for disease progression, one (3%) in the 12-week arm, and 7 (11%) in the 24-week arm.

Study treatment was generally well tolerated. **Table 4** summarizes reportable toxicities per study protocol. Toxicity was not common and included in the 24 weeks arm: Grade 4 elevated liver function tests in one patient (2%), which was considered as a serious event possibly related to

study treatment; grade 3 diarrhea in one patient (2%); and grade 3 mucositis in one patient (2%). The 12 weeks arm had one grade 3 anemia (3%) and one grade 3 renal calculi (3%). The latter was the other serious adverse event in the primary cohort and was not related to study medication.

Discussion

The introduction of anti-HER2 therapy has led to dramatic improvements in the outcome of patients with HER2-positive breast cancer. In the neoadjuvant setting, dual HER2 targeting increases pCR rates with chemotherapy (9-11).

TBCRC006 demonstrated a 27% pCR rate despite the large median tumor size of 6 cm (9). Moreover, 22% of patients had residual in-breast disease of 1 cm or less (33% in patients whose tumors were ER-positive (9)). It was therefore reasonable to hypothesize that longer treatment would result in higher pCR rate especially in the ER-positive subgroup, and this was the hypothesis tested in TBCRC023. The 24-week arm was powered to detect an increase in pCR based on a historical control (TBCRC006), and numeric superiority but not direct comparison to the 12-week arm. pCR rate was lower than expected in both study arms, and as such the study did not meet its primary endpoint. Despite that fact, our findings showed that longer duration of therapy (24 weeks) resulted in a numerically higher pCR rate in patients with HER2-positive and ER-positive disease (33% vs 9%, a difference of 24% (95% CI 5.3% to 42.3%)).

Symmans *et al*., demonstrated that patients with small amounts of residual disease (RCB-I) have a less favorable outcome than those with pCR (RCB-0) in response to chemotherapy plus anti-

HER2 therapy (18). Our results suggest that longer duration therapy may convert some cases with small amounts of residual disease into pCRs. Our findings also argue that optimal duration of therapy for different subgroups (ER-positive vs ER-negative) in HER2-positive breast cancer are different, and that the ER-positive subset appears to benefit from a longer duration of therapy. This is also supported by findings from other recent adjuvant therapy clinical trials in HER2-positive breast cancer. The ExteNET trial showed that the ER-positive subgroup appears to derive a larger benefit from longer duration of HER2-targeted therapy with the sequential use of neratinib after trastuzumab for a total of 24 months (19).

In the past few years, several chemotherapy-based neoadjuvant studies reported an increased rate of pCR with dual anti-HER2 therapy over single agent anti-HER2 therapy (11-14). The second generation of adjuvant HER2-targeted trials have thus far showed only a modest improvement in survival with the addition of a second anti-HER2 agent to trastuzumab, and this is in great part due to the inability to enrich traditional postoperative adjuvant trials with the patients most likely to benefit from such strategies. Hence, there are now significant efforts to design trial enrichment strategies that help more carefully select patients who might be candidates for escalation and de-escalation strategies, such as those pursued in our studies of HER2-targeted therapy without chemotherapy and other trials (10, 13, 20, 21).

The increase in pCR with prolonged treatment needs to be weighed against a higher progression rate in the 24-week arm over the 12-week arm (11% vs 3%). This supports the need to develop a strategy to select or enrich for patients who are likely to benefit from de-escalation, and in whom chemotherapy may be safely omitted without compromising outcomes.

Identifying the patient population who may benefit from de-escalated treatment may primarily depend on the biology of each tumor. However, equally important is the consideration of patient characteristics since some patients, particularly the elderly and patients with comorbidities may be less tolerant to chemotherapy. These patients may be good candidates for de-escalated treatment approaches. Such approaches may also be studied in lower risk tumors (*e.g.,* stage I disease) where outcomes are very favorable and less toxic regimens may be desirable. These patients are generally considered good candidates for de-escalation, as seen in the APT trial (22). Interestingly, the WSG-ADAPT trial testing T-DM1 in early HER2-positive/ER-positive breast cancer, with or without endocrine therapy and no systemic chemotherapy, reported an overall pCR rate of >40%, although adding endocrine to T-DM1 did not offer added benefit (23). While these trials suggest the clinical prospect of de-escalated treatment regimens, currently chemotherapy has a prominent and undeniable role in disease management, when considering the entire population of HER2-positive breast cancer. As such, patients who have higher clinical risk disease or unfavorable biology, who would probably benefit from chemotherapy should receive it. Treatment strategies therefore need to be tailored to the clinical and biologic characteristics of each tumor and each patient. One size does not fit all.

HER2-positive breast cancer is increasingly being recognized as a heterogeneous collection of tumors with differential responses to HER2-targeted therapy. Characterizing the molecular and biologic heterogeneity of HER2-positive breast cancer through correlative studies using tumor specimens from chemotherapy-sparing clinical trials, such as ours, will help identify the subset of tumors that are truly dependent on HER2 and will therefore benefit the most from HER2-targeted therapy alone. Intrinsic subtype, deregulation of signaling pathways like PI3K, and immune infiltrates have been linked to treatment response and resistance (10, 13, 24, 25). Indeed,

our recent TBCRC006 correlative biomarker study showed that high HER2 amplification levels combined with intact PI3K pathway, defined by wild-type *PIK3CA* or high PTEN expression, identifies a subset of HER2-positive breast cancers that may be sensitive to HER2-targeted therapy alone, without chemotherapy (26). Further, in a parallel correlative RNA-based study using tumors from 3 consecutive neoadjuvant trials in HER2-positive breast cancer with lapatinib plus trastuzumab, but without chemotherapy, including the TBCRC023, we have recently shown in tumors defined as HER2-positive by standard methods, that the HER2-enriched subtype (by nCounter PAM50) together with high *ERBB2* mRNA levels predict higher pathologic response compared to HER2-positive tumors that are not HER2-enriched and/or do not have high *ERBB2* mRNA levels (27, 28). Greater knowledge about these molecular and pathological features are therefore expected to influence the design of future clinical trials.

Our trial has limitations in that it was not powered for a direct comparison between treatment arms and its results need to be validated in an adequately powered larger trial. However, if validated,our data suggest that longer duration of treatment with targeted therapy alone (along with endocrine therapy if ER-positive) without chemotherapy increases the likelihood of pCR, especially in ER-positive patients. Correlative studies on tissue samples obtained over the course of this trial will help elucidate the mechanisms of sensitivity and resistance and will influence the next generation of clinical trials that employ a molecular triage approach for de-escalation of treatment. Identifying upfront those patients who may be spared chemotherapy and those who may benefit from it will lead to more tailored treatment recommendations based on the biology of the disease, and appropriate therapy escalation or de-escalation.

FIGURE LEGENDS:

Figure 1. **A**) **Study schema of the TBCRC023 clinical trial**. Patients with HER2-positive breast tumors measuring >2 cm received lapatinib and trastuzumab with letrozole when ER-positive (+), but without chemotherapy, for either 12 or 24 weeks. Biopsies were collected at baseline (before treatment), and after 1 week and at the time of surgery (or completion of study treatment). Tumor biopsies were also collected at 12 weeks from patients on the 24-week arm, if they had residual tumor. **B**) **CONSORT diagram of the TBCRC023 clinical trial**.

Figure 2. **Pathologic responses in the 12 vs. 24 week treatment arms by ER status.** Stacked bar graphs showing the percentage of patients with pathologic complete response (*Blue*) and minimal residual disease (<1cm, *red*) in the 12 vs. 24 week treatment arms by tumor ER status.

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References:

1. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989;244(4905):707-12.
2. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE, Jr., 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):3366-73.
3. Gianni L, Dafni U, Gelber RD, Azambuja E, Muehlbauer S, Goldhirsch A, 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. The lancet oncology. 2011;12(3):236-44.
4. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011;365(14):1273-83.
5. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol. 2006;7(7):505-16.
6. Arpino G, Gutierrez C, Weiss H, Rimawi M, Massarweh S, Bharwani L, et al. Treatment of human epidermal growth factor receptor 2-overexpressing breast cancer xenografts with multiagent HER-targeted therapy. Journal of the National Cancer Institute. 2007;99(9):694-705.
7. Rimawi MF, Wiechmann LS, Wang YC, Huang C, Migliaccio I, Wu MF, et al. Reduced dose and intermittent treatment with lapatinib and trastuzumab for potent blockade of the HER pathway in HER2/neu-overexpressing breast tumor xenografts. Clin Cancer Res. 2011;17(6):1351-61.
8. Wang YC, Morrison G, Gillihan R, Guo J, Ward RM, Fu X, et al. Different mechanisms for resistance to trastuzumab versus lapatinib in HER2-positive breast cancers--role of estrogen receptor and HER2 reactivation. Breast cancer research : BCR. 2011;13(6):R121.
9. Rimawi MF, Mayer IA, Forero A, Nanda R, Goetz MP, Rodriguez AA, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. J Clin Oncol. 2013;31(14):1726-31.
10. Llombart-Cussac A, Cortes J, Pare L, Galvan P, Bermejo B, Martinez N, et al. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. The lancet oncology. 2017;18(4):545-54.
11. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, 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.
12. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet. 2012;379(9816):633-40.
13. Carey LA, Berry DA, Cirrincione CT, Barry WT, Pitcher BN, Harris LN, et al. Molecular Heterogeneity and Response to Neoadjuvant Human Epidermal Growth Factor Receptor 2

Targeting in CALGB 40601, a Randomized Phase III Trial of Paclitaxel Plus Trastuzumab With or Without Lapatinib. J Clin Oncol. 2016;34(6):542-9.
14. Robidoux A, Tang G, Rastogi P, Geyer CE, Jr., Azar CA, Atkins JN, 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):1183-92.
15. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007;25(1):118-45.
16. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Archives of pathology & laboratory medicine. 2010;134(6):907-22.
17. Jung SH, Lee T, Kim K, George SL. Admissible two-stage designs for phase II cancer clinical trials. Stat Med. 2004;23(4):561-9.
18. Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-Term Prognostic Risk After Neoadjuvant Chemotherapy Associated With Residual Cancer Burden and Breast Cancer Subtype. J Clin Oncol. 2017;35(10):1049-60.
19. Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(12):1688-700.
20. von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. The New England journal of medicine. 2017.
21. Piccart-Gebhart M.J HAP, Baselga J., De Azambuja E., Dueck A.C., Viale G., Zujewski J.A., Goldhirsch A., Santillana S., Pritchard K.I., Wolff A.C., Jackisch C., Lang I., Untch M., Smith I.E., Boyle F., Xu B., Gomez H.L., Gelber R.D., Perez E.A., editor First results from the phase III ALTTO trial (BIG 2-06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone (L), trastuzumab alone (T), their sequence (T→L), or their combination (T+L) in the adjuvant treatment of HER2-positive early breast cancer (EBC). American Society of Clinical Oncology Annual Meeting; 2014; Chicago.
22. Tolaney SM, Barry WT, Dang CT, Yardley DA, Moy B, Marcom PK, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. N Engl J Med. 2015;372(2):134-41.
23. Harbeck N, Gluz O, Christgen M, Braun MW, Kummel S, Potenberg J, et al. Efficacy of 12-weeks of neoadjuvant TDM1 with or without endocrine therapy in HER2-positive hormone-receptor-positive early breast cancer: WSG-ADAPT HER2+/HR+ phase II trial. Journal of Clinical Oncology. 2015;33(15_suppl):506-.
24. Rimawi MF, De Angelis C, Contreras A, Pareja F, Geyer FC, Burke KA, et al. Low PTEN levels and PIK3CA mutations predict resistance to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2 over-expressing breast cancer. Breast Cancer Res Treat. 2018;167(3):731-40.

25. Loibl S, Majewski I, Guarneri V, Nekljudova V, Holmes E, Bria E, 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. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2016;27(8):1519-25.
26. Veeraraghavan J, De Angelis C, Mao R, Wang T, Herrera S, Pavlick AC, et al. A combinatorial biomarker predicts pathologic complete response to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2+ breast cancer. Ann Oncol. 2019;In Press.
27. Prat A, Angelis CD, Pascual Ts, Gutierrez C, Llombart-Cussac A, Wang T, et al. HER2-enriched subtype and ERBB2 mRNA as predictors of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer: A combined analysis of TBCRC006/023 and PAMELA trials. Journal of Clinical Oncology. 2018;36(15_suppl):509-.
28. Prat A, Pascual T, De Angelis C, Gutierrez C, Llombart-Cussac A, Wang T, et al. HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. Journal of the National Cancer Institute. 2019.

Table 1: Tissue Collection Rates (n=94)

Time Point	n	%
Baseline biopsy	94	100%
Week 1 biopsy	85	90%
Week 12 biopsy	60	64%
Surgery	78	83%

Table 2: Patient Characteristics

		All (n=97)		12-week arm (n=33)		24-week arm (n=64)	
		n	%	n	%	n	%
Age, years							
	<=50	41	42%	10	30%	31	48%
	>50	56	58%	23	70%	33	52%
	Median, Range	51	23 - 80	55	38 - 75	50	23 - 80
Race							
	White	75	77%	24	73%	51	80%
	Black	17	18%	7	21%	10	16%
	Asian	4	4%	2	6%	2	3%
	Unknown	1	1%			1	2%
Ethnicity							
	Hispanic	19	20%	8	24%	11	17%
	Not Hispanic	77	79%	24	73%	53	83%
	Unknown	1	1%	1	3%		
Menstrual Status							
	Postmenopausal	53	55%	20	61%	33	52%
	Premenopausal	44	45%	13	39%	31	48%
Tumor Size							
	<=5cm	57	60%	16	48%	41	66%
	>5cm	38	40%	17	52%	21	34%
	missing	2				2	
	Median, Range	5 cm	0 - 15	5.5 cm	2.4 - 15	4.5 cm	0 - 13
Clinical Stage							
	IIA	36	37%	12	37%	24	38%
	IIB	31	32%	9	27%	22	34%
	IIIA	16	16%	7	21%	9	14%
	IIIB	13	13%	5	15%	8	13%
	IIIC	1	1%			1	2%
Histopathologic Type							
	IDC	94	97%	32	97%	62	97%
	ILC	2	2%	1	3%	1	2%
	Other	1	1%			1	2%
Histologic grade							
	I	1	1%	1	3%		
	II	27	28%	10	30%	17	27%
	III	69	71%	22	67%	47	73%
Inflammatory							
	No	94	97%	30	100%	61	95%
	Yes	3	3%			3	5%
ER							
	+	63	65%	23	70%	40	63%
	-	34	35%	10	30%	24	38%
PR							
	+	51	53%	19	58%	32	50%
	-	46	47%	14	42%	32	50%
HER2							
	+	97	100	33	100%	64	100%

Table 3: Pathologic response rates

Pathologic complete response (pCR, ypT_{0-is})	12 weeks	24 weeks
Overall	**4/33 (12%)**	**17/61 (28%)**
ER-positive	2/23 (9%)	13/39 (33%)
ER-negative	2/10 (20%)	4/22 (18%)

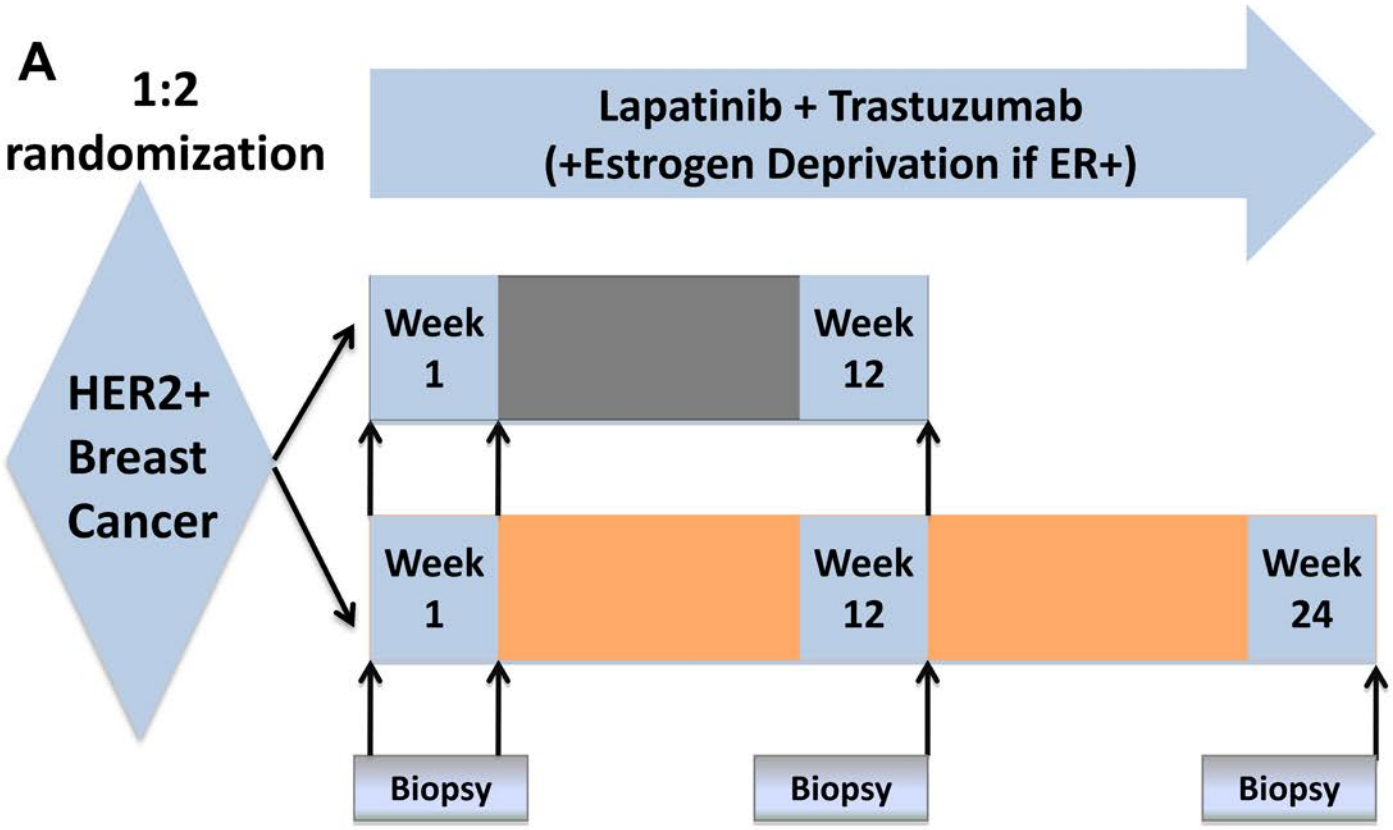
pCR, pathological complete response; ypT0-is, no residual invasive carcinoma in the breast; ER, estrogen receptor.

Table 4: Targeted Adverse Events

Grade 3 Toxicity	12 weeks (n=33) N (%)	24 weeks (n=64) N (%)
Elevated LFT (grade 4, SAE)	–	1 (2%)
Diarrhea	–	1 (2%)
Mucositis	–	1 (2%)
Anemia	1 (3%)	–
Renal calculi (SAE)	1 (3%)	–

SAE, Serious adverse events

Figure 1: Study schema and CONSORT diagram of the TBCRC023 trial



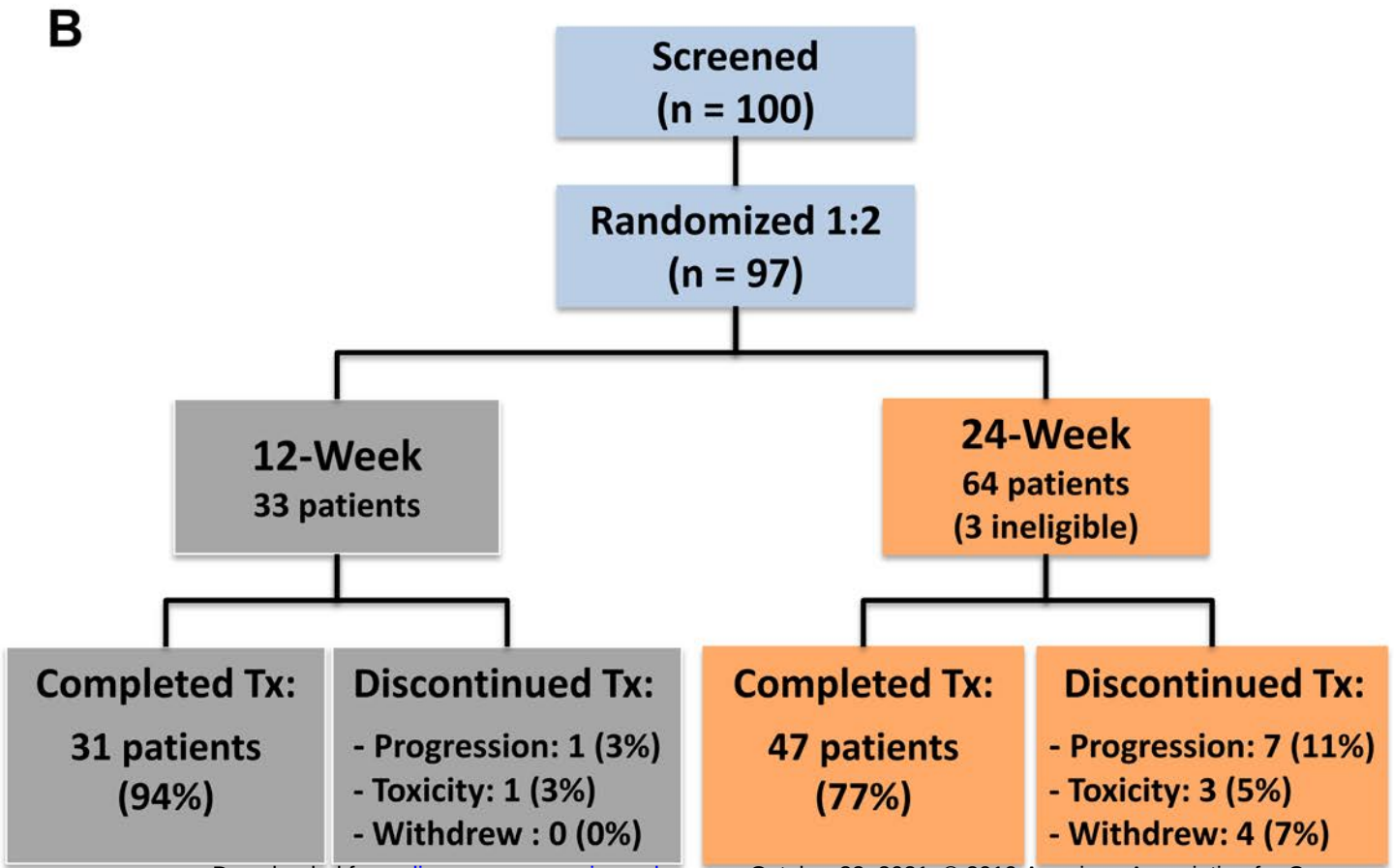


Figure 2: Pathologic Response by treatment arm and ER status