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TBCRC 008: Early change in 18-fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) predicts response to preoperative systemic therapy (PST) in HER2-negative primary operable breast cancer

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Running head: PET as a Biomarker in Breast Cancer

Abstract

Epigenetic modifiers, including the histone deacetylase inhibitor vorinostat, may sensitize tumors to chemotherapy and enhance outcomes. We conducted a multicenter randomized phase II neoadjuvant trial of carboplatin and nanoparticle albumin-bound paclitaxel (CP) with vorinostat or placebo in women with stage II/III, HER2-negative breast cancer, in which we also examined whether change in maximum standardized uptake values corrected for lean body mass (SULmax) on FDG-PET predicted pathologic complete response (pCR) in breast and axillary lymph nodes.

Methods: Participants were randomly assigned to 12 weeks of preoperative carboplatin (AUC 2 weekly) and *nab*-paclitaxel (100 mg/m^2 weekly) with vorinostat (400 mg oral daily, days 1-3 of every 7-day period) or placebo. All patients underwent FDG-PET and research biopsy pretreatment and on C1D15. The primary endpoint was the pCR rate. Secondary objectives included correlation of change in tumor SULmax on FDG-PET by C1D15 with pCR, and to correlate baseline and change in Ki67 with pCR.

Results: In an intent-to-treat analysis (n=62), overall pCR was 27.4% (vorinostat 25.8%, placebo 29.0%). In a pooled analysis (n=59), we observed a significant difference in median change in SULmax 15 days after initiating preoperative therapy between those achieving pCR versus not (percent reduction 63.0% vs. 32.9%; P=0.003). Patients with ≥50% reduction in SULmax were more likely to achieve pCR, which remained statistically significant in multivariable analysis including estrogen receptor status (OR=5.1; 95% CI=1.3-22.7; P=0.023). Differences in baseline and change in Ki67 were not significantly different between those achieving pCR versus not.

Conclusion: Preoperative CP with vorinostat or placebo is associated with similar pCR rates. Early change in SULmax on FDG-PET 15 days after initiating preoperative therapy has potential in predicting pCR in patients with HER2-negative breast cancer. Future studies will further test FDG-PET as a potential treatment-selection biomarker.

Key Words: neoadjuvant, breast cancer, FDG-PET, biomarker, vorinostat

INTRODUCTION

Chemotherapy is currently the only standard (neo) adjuvant treatment for women with triple-negative (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative, human epidermal growth factor receptor 2 [HER2]-negative) breast cancer, and is also administered to women with the luminal B subtype prior to endocrine therapy. Many women suffer recurrence and death despite this approach, emphasizing the need for new therapeutic strategies. Preclinical research supports the investigation of epigenetic modifiers such as the histone deacetylase (HDAC) inhibitor vorinostat for the treatment of breast cancer. In ER-positive and ER-negative breast cancer cell lines, vorinostat reduces proliferation, induces cell cycle arrest and modulates gene expression (*1*) and enhances the cytotoxicity of chemotherapy in in vitro models (*2*). Phase 1 investigation of the combination of vorinostat with carboplatin and paclitaxel was well tolerated and yielded a recommended phase II dose (RP2D) (*3*).

Prognostic and treatment-selection biomarkers for breast cancer patients, that could be determined at baseline or following a short duration of therapy, are also needed. Preoperative systemic therapy (PST) is an attractive model for the assessment of novel therapeutic agents, and surrogate biomarkers of response (*4*). Pathologic complete response (pCR) following PST is an accepted primary endpoint in clinical trials, predicting improved disease-free and overall survival (*5-7*). Several biomarkers have been proposed that may predict pCR, including early changes in fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) (*8*), however prospective studies establishing standardized methods and defining optimal cutpoints are needed to establish the clinical utility of this tool.

We hypothesized that the addition of vorinostat would improve the pCR rate observed with PST alone in women with stage II-III, grade 2-3, HER2-negative breast cancer. We also hypothesized that changes by cycle 1 day 15 (C1D15) of therapy in tumor standardized uptake values corrected for lean body mass (SULmax) on FDG-PET, and the proliferation index Ki67, would predict pCR. To test these hypotheses we performed a double-blind, randomized, placebo-controlled multicenter phase II study of 12 weeks of preoperative carboplatin and nanoparticle albumin-bound (*nab*) paclitaxel (CP) with vorinostat or placebo, incorporating early tissue and FDG-PET imaging biomarker evaluations (NCT00616967).

MATERIALS AND METHODS

Eligibility

Women 18 years of age or older, with histologically-proven infiltrating carcinoma of the breast were eligible if they presented with operable, clinical stage T1c, N1-3 or T2-4 lesions, any N; and M0. Tumors must have been HER2-negative and grade 2 or 3, with any ER or PR status. Eastern Cooperative Oncology Group (ECOG) performance status 0-1, and adequate organ function were required. Exclusion criteria were outlined in the clinical protocol. Women signed an informed consent approved by the Institutional Review Boards of participating institutions.

Study Design

Prior to initiating the multicenter, double-blind, randomized phase II portion of the study, we conducted a non-randomized, single-site open-label safety run-in phase. Participants received 12 weeks of preoperative carboplatin (AUC 2 weekly) and *nab*-paclitaxel (100 mg/m^2 weekly) with vorinostat (400 mg oral daily, days 1-3 of every 7-day period). Statistical considerations were included in the research protocol.

Participants in the phase II portion of the study were randomly assigned 1:1 to receive CP with vorinostat or placebo using permuted block randomization, stratified by hormone receptor status (ER and PR < 1% vs. ER or PR ≥1%). Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 was used to grade toxicity. Additional non-study chemotherapy (doxorubicin and cyclophosphamide, AC, per guidelines) was allowed per treating physician discretion prior to definitive surgery, for patients with incomplete response or disease progression on study treatment. Tumor biopsy and blood sample was requested prior to non-study chemotherapy.

Prophylactic growth factor support and dose modifications for toxicity were suggested in the research protocol. Axillary evaluation before initiating study therapy and final surgery type were per treating surgeon discretion. Administration of postoperative radiation and systemic therapies were also per discretion of the treating team with guidelines in the research protocol.

^{18}F-FDG PET/CT

Participants underwent FDG-PET/CT from mid-skull to mid-femur level prior to tumor biopsy, as previously described, at baseline and C1D15 (*9*). Each site was asked to acquire and reconstruct a very specific phantom study for central site review. That phantom was reviewed for a variety of quantitative features (e.g. uniformity, Max & Mean standardized uptake values [SUV] values, etc.) before the site was qualified for participation in the trial. Each site has also submitted a whole-body FDG-PET clinical study example for review by a physicist at the central site prior to site's acceptance into study. A uniform imaging protocol was provided to all users which included dosing (0.22 mCi/kg ±20%), uptake (60 minutes ±10 mins), plasma glucose recording. Imaging was not performed if plasma glucose was > 200 mg/dL. The protocol was

based on our own clinicians' FDG-PET/CT protocol with components drawn from published protocols as well as institutional experience (*10*).

Regions of interest (ROIs) were captured over the entire volume of disease tissue and primary tumor. SULmax was collected as it is more consistent in absolute value than SUV from patient to patient in normal tissues, being less weight dependent (*11*). Imaging and quantitation were centrally reviewed.

Immunohistochemistry

A study-specific core biopsy was obtained at baseline, C1D15, prior to non-study pre-operative chemotherapy if given, and at the time of definitive surgery. Tumor biopsy at baseline and C1D15 was performed preferably about four hours following vorinostat dose and prior to carboplatin and *nab*-paclitaxel dose. Slides were stained for Ki67 and ER using commercially available monoclonal antibodies (Ki67: Immunotech, Westbrook, ME; ER: Leicabiosystems, Newcastle UK, clone 6f11) in a CLIA-certified laboratory, and centrally quantified by the study pathologist.

Statistical Considerations

The primary end point was pCR, defined as no viable invasive cancer in breast and axilla. All other cases were defined as non-pCR. The pCR rate was determined in each arm separately by performing an intent-to-treat (ITT) analysis of all randomized patients. Patients with unknown pCR status were considered pathologic non-responders. Computation of associated 90% confidence intervals did not account for the sequential design.

The study included a concurrent randomized control arm of CP plus placebo for the primary endpoint. The design required 31 women per arm to detect a 25% pCR rate from null response rate of 10% using Simon two-stage design with 80% power and 10% Type I error rate.

There was no intention to formally compare the two arms. A single interim analysis for futility was performed once 16 patients underwent surgery in each arm by the study statistician and an investigator independent of the study conduct, both masked to treatment. A preplanned blinded interim analysis for toxicity was conducted in the first 24 patients.

Key secondary objectives were to correlate change in tumor SULmax on FDG-PET by C1D15 with pCR, and to correlate baseline and change in Ki67 with pCR. Percent reduction in SULmax treated as a continuous variable was compared between responders and non-responders using nonparametric Wilcoxon rank sum test, and Fisher's exact test when dichotomized at a pre-defined threshold (≥50% reduction). A Receiver Operating Characteristics (ROC) curve analysis was performed to explore the best cutoff of SULmax reduction and its predictive accuracy for pCR. Association of baseline and change in SULmax or Ki67 with pCR was evaluated using logistic regression models, with adjustment for hormone receptor status in multivariable analysis. Additional unplanned post-hoc analyses, performed for both primary and secondary objectives, considered patients who received additional therapies prior to surgery as pathologic non-responders.

Safety analysis included patients who received at least one dose of any study drug. All quantitative parameters were expressed as mean ± standard deviation (SD) or median and range. All statistical tests were two-sided and considered statistically significant at $P<0.05$. The analyses were carried out using SAS (version 9.2, SAS Institute, Cary, NC) and R software packages (version 2.15.2). The research protocol and manuscript were written by the authors and reviewed by the pharmaceutical funders, who had no access to the study database and were not involved in study analysis or interpretation of results.

RESULTS

Safety Run-in

Six patients were enrolled in the safety run-in phase. The combination was well tolerated with predominantly grade 1 and 2 adverse events. The RP2D for CP was as described above, in combination with 400 mg of vorinostat or placebo.

Phase II

Patient Characteristics. From October 2009 to November 2011, 62 women enrolled in the study with patient characteristics well balanced across treatment groups (Table 1). Of the 62 randomized, 61 completed study drugs and 60 completed primary surgery. Two patients with unknown pCR status were regarded as pathologic non-responders on an ITT basis. Eighteen of 60 women (6 vorinostat arm, 12 placebo arm) also received preoperative non-study chemotherapy (anthracycline-based) due to incomplete clinical response or physician preference (including 8 women with clinical complete response), and were included in the ITT analysis (Figure 1). Dose modifications are described in Supplementary Table 1. Thirty three patients received AC, and two received cisplatin, postoperatively

Treatment Safety. Hematological and non-hematological toxicities are shown in Supplementary Table 2. There were no significant differences in adverse events between the arms. The preplanned blinded interim toxicity analysis did not meet the criteria for early stopping.

Treatment Efficacy. Overall, pCR was observed in 17 patients (27.4%; 95% CI = 16.9% - 40.2%) in the ITT population, meeting the predefined aim of 25% in each arm; 8 in vorinostat arm (25.8%; 95% CI: 11.9%-44.6%) and 9 in placebo arm (29.0%; 95% CI: 14.2% – 48.0%). As the pCR rates in both arms were similar, we pooled the arms to obtain this overall pCR rate with

a 95% confidence interval. Of patients obtaining pCR, 12 were ER and PR-negative, 5 were ER or PR-positive (2 with known BRCA mutations, one in each arm, one whom received pre-operative non study chemotherapy) and all were grade 3. When stratified by hormone receptor status, the pCR rate in the placebo arm was 10.5% (2/19; 95% CI: 1.3% – 33.1%) and in the vorinostat arm was 15.8% (3/19; 95% CI: 3.4% – 39.6%) for patients with ER or PR-positive disease; 58.3% (7/12; 95% CI: 27.6% – 84.8%) in the placebo arm and 41.7% (5/12; 95% CI: 15.2% – 72.3%) in the vorinostat arm for patients with triple-negative breast cancer (Figure 2). Additionally, we performed an analysis considering patients who received additional non-study preoperative chemotherapy as pathologic non-responders, to provide a conservative estimate for pCR rate with study therapy alone. The overall pCR rate was 17.7% (11/62; 95% CI = 9.2% - 29.5%); 16.1% for vorinostat arm (5/31; 95% CI: 5.5%-33.7%) and 19.4% for placebo arm (6/31; 95% CI: 7.5% – 37.5%).

Baseline and Change in Biomarkers and Correlation with Response to Therapy. All 62 patients underwent baseline study biopsy and FDG-PET, and 59 (95.2%) and 61 (98.4%) underwent both baseline and C1D15 biopsy and FDG-PET, respectively. Baseline FDG-PET occurred within 30 days prior to initiation of study therapy (75% within one week of initiation of therapy, range 0-30) and C1D15 FDG-PET occurred on D15 in 66% of cases (range D14-22). FDG-PET was performed prior to study biopsy in all but 4 cases (baseline), and in all cases at C1D15. No significant differences in the biomarkers were found between treatment groups, and thus a pooled analysis was performed using data from patients with valid FDG-PET data at baseline and C1D15. Change in SULmax was evaluated in 59 women (16 pCR, 43 no pCR), where 3 were not evaluable (technically invalid FDG-PET data [n=2], or no available C1D15 FDG-PET [n=1]). Median baseline SULmax in pathologic responders was significantly higher vs.

pathologic non-responders (7.6 vs. 5.3; P=0.018; Table 2). A significant difference in change in SULmax was observed between pathologic responders and non-responders (median percent reduction 63% vs. 33%; P=0.003; Figure 3 and Table 2). Seventy-five percent of pathologic responders exhibited ≥ 50% reduction in SULmax at C1D15 vs. 30% of non-responders (P=0.003; Table 2). Patients with ≥50% reduction in SULmax were more likely to achieve a pCR, as suggested by both univariate (OR=6.6; 95% CI=1.9-27.3; P=0.004) and multivariable analyses adjusting for hormone receptor status (OR=5.1; 95% CI=1.3-22.7; P=0.023; Table 3). By ROC analysis, discrimination between patients with pCR (pathologic responders) and no pCR (pathologic non-responders) resulted in an area under the curve (AUC) of 0.76 (95% CI=0.60-0.91) with sensitivity of 0.75 and specificity of 0.74 at a cutoff of 52% reduction in SULmax that maximized the sum of sensitivity and specificity. Negative predictive value (NPV) and positive predictive value (PPV) were 89% and 52%, respectively. A post-hoc sensitivity analysis was performed excluding patients who received additional non-study preoperative therapy and demonstrated similar results (Supplementary Tables 3 and 4).

Forty-four matched specimens (baseline, C1D15) were evaluable for change in Ki67 (8 pCR, 36 no pCR); non-evaluable samples had no tumor cells present or Ki67 unavailable at either or both time points. We did not observe statistically significant differences in baseline and C1D15 percent change in Ki67 between pathologic responders and non-responders, nor a significant change in ER status between pre and post therapy (Supplementary Table 5).

DISCUSSION

We demonstrated that preoperative CP plus vorinostat or placebo was associated with similar pCR rates in women with HER2-negative breast cancer. The pCR rate observed overall

in the ITT population (27%) was greater than initially predicted based on historical data in unselected breast cancer subtypes (*12*). However, a proportion of patients received additional non-study preoperative chemotherapy per physician discretion, which likely had an impact on the assessment of the primary endpoint. pCR was observed only in women with grade 3 tumors and predominantly in those with triple-negative disease. Interestingly, of those with ER or PR-positive tumors who experienced a pCR, 2 patients had BRCA mutations, suggesting that CP may warrant further evaluation in this population. These results are reflective of the high pCR rate observed, irrespective of hormone receptor status, in a recent study of neoadjuvant cisplatin in patients with BRCA1 mutations (*13*).

Early change in SUV uptake on FDG-PET has been investigated as a predictor of response to PST (*8*). In a prospective trial in which 104 patients with large (≥ 3cm) or locally advanced breast cancer were randomized to two anthracycline/taxane-based neoadjuvant chemotherapy regimens, FDG-PET scans were obtained at baseline and after one and two cycles of chemotherapy (*14*). A threshold of 45% decrease in SUV after the first cycle of chemotherapy correctly identified 11 of 15 patients who obtained a pCR, and those who did not obtain a pCR were identified with a negative predictive value of 90%. After the second cycle of chemotherapy, a threshold of 55% relative decrease in SUV predicted pCR. We enrolled a similar patient population and all participants received a relatively homogeneous chemotherapy. A meta-analysis of 19 studies and 920 patients with pCR aimed to predict pathological response in primary breast lesions by FDG-PET (*15*). The best correlation with pCR employed a 55-65% reduction rate cutoff value of SUV. An exploratory ROC analysis from our study indicated that a 52% reduction in SULmax predicted pCR with sensitivity 75%, specificity 74%, NPV 89% and PPV 52% (AUC 0.76, 95% CI=0.60-0.91). Other studies performed to date have been

predominantly retrospective, and associated with significant heterogeneity of the breast cancer subtypes investigated, chemotherapy administered, timing of imaging in relation to chemotherapy, and definitions of response to therapy (*14,16-19*). The significant difference we observed in median percent reduction in SULmax on FDG-PET between pathologic responders and non responders in a pooled analysis with a relatively large odds ratio suggests that SULmax is a promising biomarker for early prediction of treatment response to this regimen. Coupled with others, our results provide valuable data regarding the optimal cut point of reduction in SULmax which can be used in future studies designed to assess whether altering therapy based on early changes will be associated with improved pCR. This study was designed before the PERCIST 1.0 PET response criteria were published, but it is expected that SULpeak changes would provide similar results, possibly with greater precision than SULmax (*11*).

Strengths of our study include the multicenter prospective randomized and placebo-controlled design. All 62 patients had baseline FDG-PET and biopsy, with almost all patients undergoing serial FDG-PET scans and biopsies; an invaluable resource for correlative analyses. Study sites were provided with FDG-PET imaging protocol recommendations to achieve a high level of multi-site consistency. This consistency was documented by few cases with uptake periods outside the specified range of 50-70 minutes post-injection (n=10/62), only three patients with blood sugar readings at the time of imaging in excess of 150 mg/dL, though all were below 200 mg/dL, and different scanners being used between baseline and follow-up scans in only 3 of the 62 cases. It is unlikely that this impacted the study results due to the small numbers involved. Central analysis of FDG-PET was successful and 90% of images were evaluable for change in SULmax. Finally, we chose an early time point for the second FDG-PET scan (after 2 weeks) to define as early as possible in the treatment paradigm those patients who will benefit from an

alternative approach. A rapid and significant decrease in glucose metabolism as early as 8 days after the administration of one cycle of combination chemotherapy in women who responded to the regimen has been previously observed and can allow for a rapid change in therapy for women who are not expected to derive benefit (*8*). This has theoretical advantages over later time points in terms of discrimination abilities between pCR and no pCR (*11*,*20*).

Limitations of our study include that a proportion of women received additional anthracycline-based preoperative chemotherapy after study treatment, which may complicate both the evaluation of our primary endpoint and the role of FDG-PET in predicting treatment response. However, post hoc sensitivity analysis which excluded patients who received pre-operative non study chemotherapy yielded similar results (Supplementary Tables 3 and 4). Only 72% of matched samples were evaluable for Ki67 analysis, partly due to lack of viable tumor cells in C1D15 specimens which may have been due to early treatment effect. The challenges surrounding serial Ki67 measurement in tumor samples have been previously described (*21*). These results suggest that the ability to quantify and image the entire tumor by FDG-PET imaging may be an advantage over a biopsy of a small region of tumor. In a small number of cases (n = 4), baseline study biopsy was performed prior to FDG-PET which may have inflated the SULmax value due to an inflammatory response. The cost associated with serial FDG-PET imaging, as well as additional radiation exposure over and above standard of care, may also be a barrier to implementing this as a treatment-selection biomarker in the clinic. However, a non-invasive biomarker that is readily available may be preferable to a tissue-based biomarker which requires additional procedures for patients. Our study did not incorporate serial MRI which may be an alternative to FDG-PET and associated with less radiation exposure (*20*). Newer FDG-PET cameras and CT algorithms can also acquire high quality images with lower radiation doses.

CONCLUSION

Our study confirms the feasibility of conducting multicenter neoadjuvant studies that add novel agents to chemotherapy backbones, and incorporate serial tissue biopsies and quantitative imaging for biomarker development. Although our study did not indicate a benefit for the addition of vorinostat to CP, the pCR rate observed suggests a potential role for platinum agents in specific breast cancer subtypes, namely high grade triple-negative or BRCA-associated tumors. Future studies will use the optimal cut point of reduction in SULmax we have identified in order to determine whether altering therapy based on early changes in SULmax is of clinical utility.

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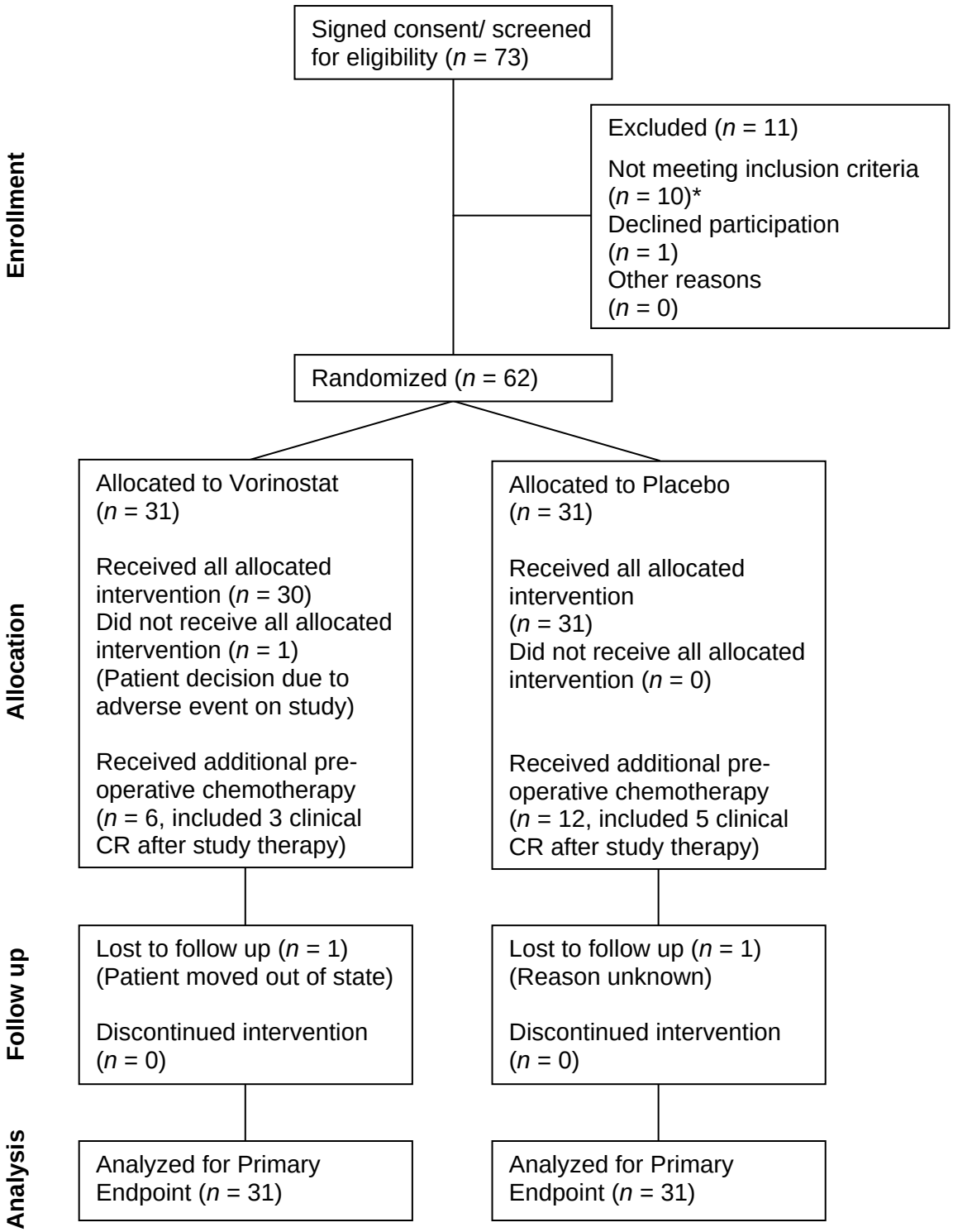

FIGURE 1: CONSORT flow diagram for Phase II. All 73 patients who signed consent for formal eligibility assessment included.

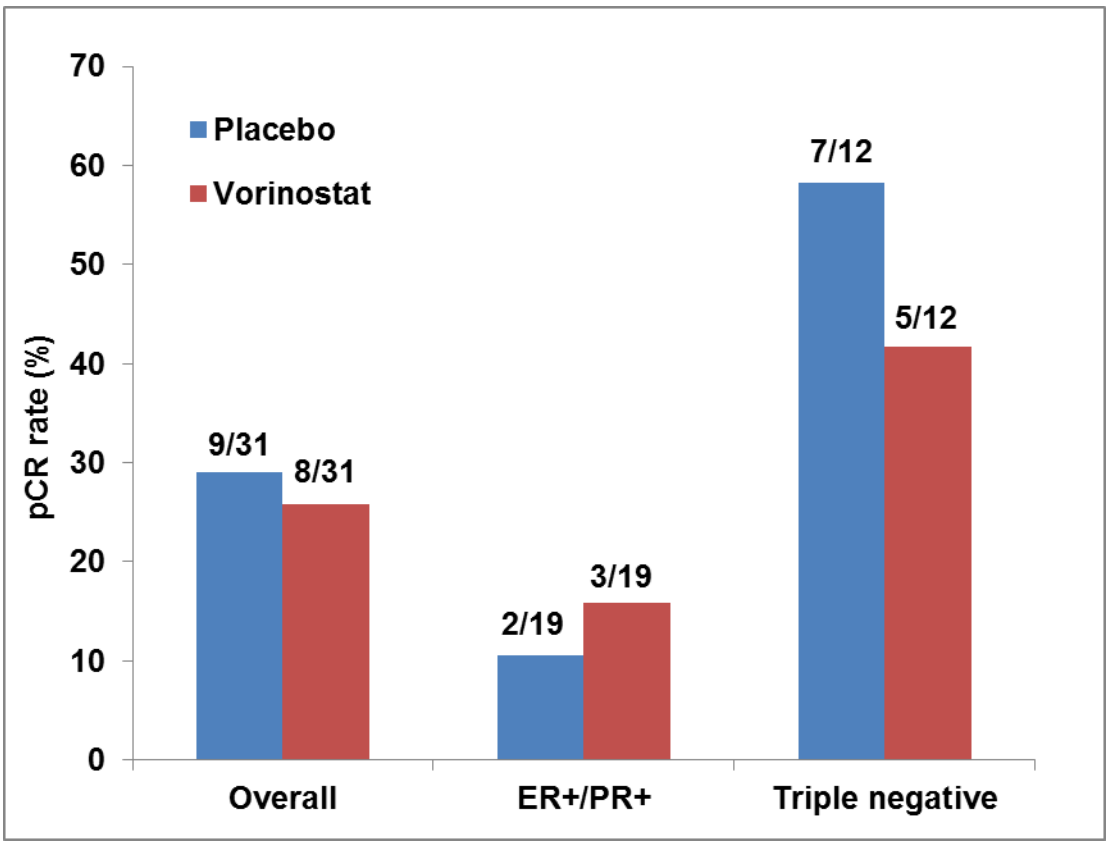

FIGURE 2: Pathological complete response (pCR) rates overall and by subgroup (intent-to-treat analysis).

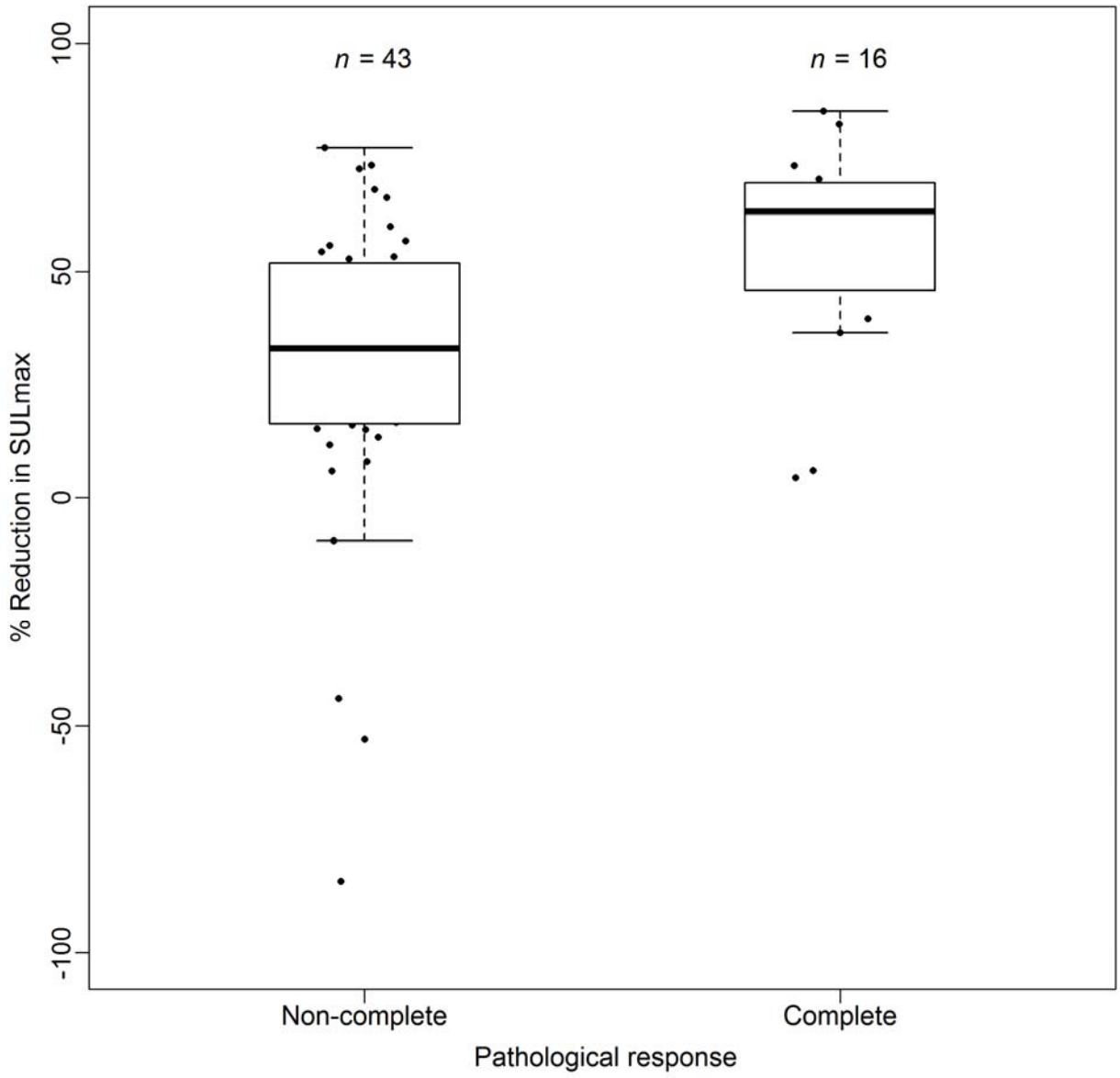

FIGURE 3: Box plots of reduction in SULmax in patients with complete and non-complete pathological response. The horizontal line inside the box shows the median. The lower and upper hinges of the box represent the 25th and 75th percentiles, respectively. The filled circles represent the actual values of percent reduction in SUL max.

TABLE 1: Patient characteristics

Characteristics	Vorinostat Arm (n=31)	Placebo Arm (n=31)	Overall (n=62)
Age, years			
Median (Range)	48 (31-68)	48 (24-72)	48 (24-72)
Race			
Caucasian	24 (77%)	19 (61%)	43 (69%)
Black	5 (16%)	8 (26%)	13 (21%)
Other	2 (7%)	4 (13%)	6 (10%)
ECOG			
0	29 (94%)	30 (97%)	59 (95%)
1	2 (6%)	1 (3%)	3 (5%)
Tumor Size (cm)			
Median (Range)	4 (1.5-11.5)	5 (1.7-18)	4 (1.5-18)
Baseline Nodal status			
Negative	14 (45%)	10 (32%)	24 (39%)
Positive	17 (55%)	21 (68%)	38 (61%)
Tumor Grade			
2	7 (23%)	11 (35%)	18 (29%)
3	24 (77%)	20 (65%)	44 (71%)
Receptor status			
ER-/PR-	12 (39%)	12 (39%)	24 (39%)
ER+/PR+	10 (32%)	12 (39%)	22 (36%)
ER+/PR-	6 (19%)	6 (19%)	12 (19%)
ER-/PR+	3 (10%)	1 (3%)	4 (6%)
BRCA status			
BRCA1/2 mutation	4 (13%)	3 (10%)	7 (11%)
BRCA negative	7 (22%)	9 (29%)	16 (26%)
Unknown	20 (65%)	19 (61%)	39 (63%)

ECOG, Eastern Co-operative Oncology Group Performance Status
ER, estrogen receptor
PR, progesterone receptor

TABLE 2: Baseline and change in SULmax between pathological responders and non-responders*

Variable	Responders (n=16)	Non-responders (n=43)	*P*#
Baseline SULmax			
Mean (± SD)	8.2 (± 3.3)	5.9 (± 3.5)	
Median (range)	7.6 (4.3 – 15.1)	5.3 (1.4 – 21.0)	0.015
%Change in SULmax			
Mean (± SD)	55.5 (± 23.6)	30 (± 32.5)	
Median (range)	63.0 (4.4 – 85.3)	32.9 (-84.2 – 77.1)	0.003
≥50% reduction, n[%]	12 (75.0)	13 (30.2)	0.003

* Pooled intent-to-treat analysis

Exact Wilcoxon rank sum test for continuous variables and Fisher's exact test for binary variables

TABLE 3: Analysis of association of SULmax with pathological response*

Variable	Univariate analysis		Multivariable analysis#	
	OR (95% CI)	*P*	Adjusted OR (95% CI)	Adjusted *P*
Baseline SULmax	1.18 (1.01–1.42)	0.051	1.07 (0.88–1.30)	0.469
%Change in SULmax	1.04 (1.01–1.08)	0.011	1.03 (1–1.07)	0.057
%Change in SULmax ≥ 50% vs. < 50%	6.6 (1.9–27.3)	0.004	5.1 (1.3–22.7)	0.023

OR = odds ratio; CI = confidence interval

* Pooled intent-to-treat analysis

Multivariable logistic regression adjusting for ER and PR status.