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Neoadjuvant trebananib plus paclitaxel-based chemotherapy for stage II/III breast cancer in the adaptively randomized I-SPY2 trial — Efficacy and biomarker discovery

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Abstract

Purpose: The neutralizing peptibody trebananib prevents angiopoietin-1 and angiopoietin-2 from binding with Tie2 receptors, inhibiting angiogenesis and proliferation. Trebananib was combined with paclitaxel+/−trastuzumab in the I-SPY2 breast cancer trial.

Patients and Methods: I-SPY2, a phase II neoadjuvant trial, adaptively randomizes patients with high-risk, early-stage breast cancer to one of several experimental therapies or control based on receptor subtypes as defined by hormone receptor (HR) and HER2-status and MammaPrint risk (MP1, MP2). The primary endpoint is pathological complete response (pCR). A therapy "graduates" if/when it achieves 85% Bayesian probability of success in a phase III trial within a given subtype. Patients received weekly paclitaxel (plus trastuzumab if HER2-positive) without (control) or with weekly intravenous trebananib, followed by doxorubicin/cyclophosphamide and surgery. Pathway-specific biomarkers were assessed for response prediction.

Results: There were 134 participants randomized to trebananib and 133 to control. Although trebananib did not graduate in any signature [phase III probabilities: HR-negative (78%), HR-negative/HER2-positive (74%), HR-negative/HER2-negative (77%), and MP2 (79%)], it demonstrated high probability of superior pCR rates over control (92%−99%) among these subtypes. Trebananib improved 3-year event-free survival (hazard ratio 0.67), with no significant increase in adverse events. Activation levels of the Tie2 receptor and downstream signaling partners predicted trebananib response in HER2-positive disease; high expression of a CD8 T cell gene signature predicted response in HR-negative/HER2-negative disease.

Conclusions: The Ang/Tie2 axis inhibitor trebananib combined with standard neoadjuvant therapy increased estimated pCR rates across HR-negative and MP2 subtypes, with probabilities of superiority >90%. Further study of Ang/Tie2 receptor axis inhibitors in validated, biomarker-predicted sensitive subtypes is warranted.

Keywords

breast cancer; clinical trial results/phase II clinical trials; translational research; precision medicine

INTRODUCTION

Angiogenesis is implicated in the development, progression and metastasis of breast and other cancers (1). The angiopoietin(Ang)-Tie2 receptor axis, in which angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) are ligands of the Tie2 receptor, plays a key role in the angiogenic switch in tumors. This is distinct from the vascular endothelial growth factor (VEGF) pathway and represents an alternate target for angiogenic inhibition (2). The two pathways promote formation and maturation of new vessels and the Ang-Tie2 axis regulates metastasis, inflammation and lymphangiogenesis (3). The biologic effects of Ang1 and Ang2 are context-dependent, functioning as either a Tie2 agonist, partial agonist or antagonist (3). The Tie2 receptor is normally expressed mostly on endothelial cells, it is also found in several other cell types, including smooth muscle cells, fibroblasts, epithelial cells, monocytes, neutrophils, eosinophils, glial cells and in macrophages. Tie2-expressing macrophages (TEM) directly and stably associate with endothelial cells and Mena-expressing tumor cells and serve as intravasation sites. This could contribute to angiogenesis and metastatic progression (4–6).

Trebananib (AMG 386, Amgen) is a peptide-Fc fusion protein that neutralizes the interaction between the Tie2 receptor and Ang1 and Ang2 (7). In preclinical studies, trebananib decreased tumor growth and induced vessel regression by inhibition of both Ang1 and Ang2 (3,8). Early data supported possible synergy with taxane-based chemotherapy. In first-inhuman studies, trebananib had a favorable toxicity profile distinct from VEGF inhibitors (9). Trebananib in combination with taxane-based chemotherapy showed increased progression-free survival but not overall survival in advanced ovarian cancer, with dose-response and exposure-response effects (10–12).

The trebananib-taxane combination was tested in metastatic breast cancer (BC) during the same time as the ovarian cancer trials. Safety data from two studies, first in HER2-negative and then subsequently in HER2-positive (13,14) breast cancer, both demonstrated good tolerance of the trebananib-taxane (+/− trastuzumab) combinations. We designed the current neoadjuvant trial in early breast cancer when this favorable safety data became available from the two ongoing metastatic trials. At the time, the trials in metastatic breast cancer had not reported efficacy yet.

I-SPY2 is an adaptively randomized neoadjuvant platform trial that evaluates investigational agents (or combinations of agents) on a background of standard-of-care therapy for stage II/III breast cancer (15–17). Herein we present the mature efficacy results of trebananib in combination with paclitaxel-based chemotherapy (+/− trastuzumab) in I-SPY2. We also report companion translational analyses that explored whether qualifying pre-specified gene-protein-phosphoprotein biomarkers in the Ang-Tie2 receptor, downstream signaling pathways and others are predictors of response to trebananib. We hypothesize that higher levels of baseline Tie2 pathway activity and associated biology within the tumor microenvironment may associate with response.

MATERIALS AND METHODS

Platform study design

I-SPY2 is an ongoing, open label, adaptively randomized phase II multicenter trial of neoadjuvant therapy for early-stage breast cancer at high risk of recurrence (clinicaltrials.gov identifier NCT01042379). As a “platform” trial, multiple investigational arms are evaluated in parallel, each consisting of an investigational agent/combination added to a backbone of standard-of-care neoadjuvant chemotherapy that also serves as a common control arm.

The primary endpoint is pathologic complete response (pCR) assessed at time of surgery, defined as the absence of invasive disease in breast and regional nodes (ypT0/is and ypN0). The primary analysis is a modified intent-to-treat, in which all participants who receive the allocated therapy are evaluable; those who switched to non-protocol assigned therapy, forwent surgery or withdrew from the trial were assigned “non-pCR” status. Secondary endpoints include residual cancer burden (RCB) (18), 3-year event-free survival (EFS) and distant relapse-free survival (DRFS). All patients are followed for long-term outcome and safety.

Hormone receptor (HR) positive or negative, HER2-positive or negative and MammaPrint (MP1 or MP2) high-risk status (Agendia, Inc. Irvine, CA) are used to classify patients into one of 8 subtypes that translate into 10 clinically relevant signatures (19). Randomization preferentially assigns patients to arms based on continuously updated Bayesian probabilities of rates of pCR for each subtype, with 20% of patients randomly assigned to the control.

An arm ‘graduates’ from I-SPY2 when, in any of the 10 clinically relevant signatures, it reaches the predefined efficacy threshold of 85% probability of success in a hypothetical, subtype-specific 300-patient, 1:1 confirmatory phase III trial. If the probability of phase III success is between 10% and 85%, the arm completes the predefined maximal accrual of 120 patients for an agent open across all subtypes. An arm is dropped for futility if the predicted probability of success in phase III is <10% in all signatures. Refer to previous publications for study design details (16,17).

Patient eligibility

Patients eligible for I-SPY2 are women ≥18 years, with stage II or III breast cancer and primary tumors >2.5 cm by clinical exam or >2.0 cm by imaging, with Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (20). Patients with tumors that are MammaPrint low-risk/HR-positive/HER2-negative are excluded from I-SPY2 as their lower risk of recurrence does not justify escalation of therapy (19,21). All patients provide written informed consent prior to screening and again after randomization.

Initially, only patients with the HER2-negative subtypes were eligible for randomization to the trebananib arm based on acceptable safety data emerging from the ongoing metastatic breast cancer trial (13). Subsequently, the HER2-positive subtypes were added to the arm by amendment when the second ongoing metastatic disease trial reported safety with trastuzumab added to taxane plus trebananib (14).

Treatment

All participants received standard of care neoadjuvant chemotherapy consisting of 80mg/m^2 intravenous paclitaxel weekly for 12 weeks, followed by four cycles of AC chemotherapy every 2–3 weeks (AC, intravenous doxorubicin, 60mg/m$^{2;}$ cyclophosphamide, 600mg/m^2). Trebananib was not continued during the AC phase. Participants with HER2-positive subtypes also received intravenous trastuzumab (4 mg/kg load in week 1, followed by 2 mg/kg weekly x 11 weeks) with the weekly paclitaxel. Patients randomized to the experimental arm received weekly infusions of 15 mg/kg trebananib for the first 12 weeks, concurrent with paclitaxel (± trastuzumab).

Definitive surgery followed AC, either a lumpectomy or mastectomy at the discretion of the treating surgeon, with either sentinel or axillary node dissection according to NCCN and local practice guidelines (22). Postoperative adjuvant treatment was not mandated by the study protocol, rather was recommended per NCCN guidelines at the discretion of the treating medical oncologist. Radiation therapy was given per national and local radiation oncology guidelines.

Assessments

Breast tumor core biopsies and breast MRIs were obtained at baseline and at 3 weeks, with an additional MRI performed between paclitaxel and AC and again following AC (16,17). Local pathologists trained in the RCB method performed the primary endpoint assessment on surgical specimens (18). Biomarkers assessed included the 70-gene MammaPrint and TargetPrint HER2 gene expression assays using the 44K full-genome microarray (Agendia) (19,23).

Prespecified ‘qualifying’ biomarkers related to trebananib’s mechanism of action were assessed using reverse-phase protein array (RPPA) based protein pathway activation mapping of laser capture microdissection (LCM) enriched tumor epithelium. The Agendia 44K full-genome microarray data was generated from pre-treatment biopsies as previously described (24). We evaluated 33 RPPA protein and phosphoprotein analytes and 14 genes/signatures involved in TIE2 signaling as biomarkers of trebananib response (see Supplementary Table S2), including direct measurement of activation/phosphorylation of the Tie2 receptor itself.

Trial oversight

The trial was designed and conducted by the I-SPY2 study investigators and sponsors, 501(c)3 Quantum Leap Healthcare Collaborative (San Francisco, CA); it complies with all local and national regulations regarding the use of human study participants and was conducted in accordance to the criteria set by the Declaration of Helsinki. The drug manufacturer, Amgen (Thousand Oaks, CA), provided funds and study drug but played no role in the study design, data collection/analysis or manuscript preparation. All participating sites received local institutional review board approval. The independent I-SPY2 Data and Safety Monitoring Board met monthly to review patient safety and study progress. The authors of the manuscript vouch for the accuracy and completeness of the data reported.

Statistical analyses

Probability distributions of pCR rates were continuously updated during the study, using a covariate-adjusted Bayesian longitudinal model based upon change in tumor volume by MRI (for those still undergoing treatment) and pathological response (for those who completed surgery) with HR, HER2 and MammaPrint statuses as covariates. The model adjusted for time trends to allow comparisons against all enrolled I-SPY2 controls prior to the date randomization stopped for the investigational arm (see Supplementary Methods). From these distributions, the probability that the pCR rate of the investigational arm was greater than control was assessed for each of the 10 clinically relevant biomarker signatures and similarly, for the predictive probabilities of success in a future 1:1 randomized 300-patient phase III trial within the responsive signature.

Kaplan-Meier (KM) EFS curves for each arm were generated, with hazard ratios (point estimates) by Cox proportional hazard modeling. Statistics regarding this exploratory EFS analysis were descriptive and not inferential, given the sample sizes were small and I-SPY2 was not powered for EFS or other survival endpoints. Similar KM curves were produced for pCR and non-PCR cohorts from the investigational and control arms.

Logistic modeling was used to assess biomarker performance in the qualifying/pre-specified biomarker analyses. A biomarker was considered a specific predictor of trebananib response if it associated with response in the trebananib arm but not the control arm, and if the biomarker-by-treatment interaction term was significant (likelihood ratio test, $p<0.05$). Qualifying biomarkers were chosen based on pre-hypothesized roles of the markers in trebananib mechanism of action (e.g. modulation of angiopoietin (Ang)-Tie2 receptor interaction). These analyses were also performed adjusting for HR/HER2 status as a covariate, and within receptor subsets, sample size permitting. Biomarkers were analyzed individually. The p-values were descriptive with no correction for multiple comparisons. All computation was performed in the R programming environment (version 3.3.3).

Data Availability Statement

Protein/phosphoprotein expression data reported in this study are available at: Gene Expression Omnibus (GEO) SubSeries GSE196093 (RPPA) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE196093), as part of the SuperSeries GSE196096 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE196096. All other deidentified subject-level clinical data reported in this manuscript may be requested by qualified investigators. Details of the trial, data, contact information, proposal forms, and review and approval process are available at the following website: https://www.ISPYtrials.org/collaborate/proposal-submissions.

RESULTS

Patient accrual and characteristics

The trebananib arm was open for randomization between October 2011 and July 2014 (Figure 1). From the start of the trial (March 2010) through trebananib arm closure, 1150 patients screened for I-SPY2 and 703 patients were randomized to one of the study arms.

Of these, 141 participants were randomized to receive trebananib (122, HER2-negative tumors; 19, HER2-positive). Over the same period, 145 participants (109, HER2-negative; 36, HER2-positive) were randomized to the standard of care control arm. Seven patients (all with HER2-negative disease) in the trebananib arm and 12 patients (five of whom had HER2-positive tumors) in the control arm did not receive the intervention allocated to them and are not included in the analysis. Thus, 134 participants received trebananib and 133 standard of care control therapy (of which 19 and 31, respectively, had HER2-positive tumors and received trastuzumab).

Characteristics of the experimental and control arm populations at baseline showed no major differences in age, race/ethnicity, HR status, HER2 status, MammaPrint high-risk status (MP1, MP2), tumor size or nodal status (Table 1). Nineteen percent in each arm were of non-White race/ethnicity (see Representativeness of Study Participant Table S1).

Efficacy

Enrollment to the trebananib arm ended upon reaching the arm's maximum accrual that included an expansion period to allow additional participants in the HER2-positive cohort after the amendment. Figure 2 shows the final probability distributions for all 10 clinical signatures. The arm did not meet the prespecified threshold for graduation (>85% predictive probability of success in a hypothetical phase III trial) in any of the 10 signatures (Figure 2, Supplementary Table S3). However, superior efficacy was suggested compared to control in several signatures: the predictive probabilities approached the 85% threshold in HR-negative (78.4%), HR-negative/HER2-positive (73.9%), HR-negative/HER2-negative (77.1%), and high MammaPrint risk MP2 (78.6%) signatures (Figure 2).

Comparisons of pCR rates versus control suggested trebananib had activity in a number of signatures (Figure 2, Supplementary Table S3). The probability that trebananib was superior to control for all signatures combined was 98.6%; HR-negative, 99.1%, high MammaPrint risk MP2, 99.1%; HR-negative/HER2-negative, 98.8%; and HR-negative/HER2-positive, 92.6%. Median follow-up for EFS was 4.8 years. As of the February, 2019 cut-off date, 125 of the 134 participants in the trebananib arm and 121 of 133 controls had complete follow-up data. In this exploratory analysis, which is presented as descriptive rather than inferential, participants in the trebananib arm had 3-year EFS of 85%, compared to 81% in the control arm, with a hazard ratio point estimate of 0.67 (Figure 3a). Among those signatures with higher probabilities of trebananib efficacy, hazard ratio point estimates were 0.45 in HR-negative (3-year EFS of 83% vs 74%, trebananib vs control, respectively); 0.41 in triple negative (3-year EFS 85% vs 74%), and 0.79, high MammaPrint risk MP2 (3-year EFS 76% vs 77%) (Figure 3B-D). We did not evaluate EFS in the HR-HER2+ due to the limited number of patients of that subtype.

Residual Cancer Burden (RCB) data were available for 258 (127 control and 131 trebananib) patients. Overall, there was a trend for a shift towards lower RCB index in the trebananib arm as compared to the control (median RCB: 1.46 vs. 1.60, Wilcoxon rank sum test $p = 0.09$). Within the HR-negative/HER2-negative signature, where the biggest differences in EFS were observed, the RCB index was significantly lower in the trebananib arm than control (median RCB: 1.06 vs. 1.57, Wilcoxon rank sum test $p = 0.006$). In keeping with the

overall I-SPY2 experience that EFS improved if pCR was achieved vs non-pCR, the 3-year EFS was superior in both trebananib and control arms, respectively, for the pCR vs non-pCR cohorts: pCR, 94% trebananib and 96% control, versus non-pCR 80% trebananib and 77%, control (Supplementary Figure S1).

Adverse events and treatment delivery

Trebananib was very well tolerated. Table 2 shows the grade 3/4 adverse events reported in 2% or greater of participants by arm by treatment phase (paclitaxel +/− trebananib followed by AC). Specifically, for trebananib plus paclitaxel, there were similar toxicities observed compared to paclitaxel alone. This was also true for the AC phases after the taxane in both arms. The only exception is that there were more neutropenia and anemia events in the AC phase after trebananib than in the AC control. There was no increase in hypertension, bleeding, or thromboembolic events with trebananib.

There were seven dose reductions (5.2%) with trebananib-paclitaxel versus two (1.5%) with paclitaxel alone (Table 2). These rates were similar in the AC phases of each arm (5.6% after trebananib and 6.3% after paclitaxel control). Early discontinuation of treatment for any reason occurred in 26 of 134 (19.4%) participants during paclitaxel + trebananib versus 22 (16.5%) during paclitaxel alone. The rates were lower during the two AC phases (4.6% and 6.3%). The most common reasons for discontinuation early were toxicity and progression of disease. The median time from treatment consent to surgery was nearly identical in the investigational and control arms: 172 and 170 days, respectively.

Biomarkers of treatment response

We assessed biomarkers in the Ang/Tie2 pathway including activation of the Tie2 receptor as predictors of response to trebananib, hypothesizing that pre-treatment/baseline levels of Tie 2 receptor activation and underpinning pathway activity may associate with response. Ang/Tie2 pathway activation was evaluated in the LCM-enriched tumor epithelium at the total protein/phospho-protein level for 33 analytes, including total and phospho-Tie2 and downstream signaling targets, and at the gene expression level for 14 genes/signatures including ANG1/2 and TIE2; 128 and 119 patients in the trebananib and control arms, respectively had enough material for RPPA analysis. Expression data was available for analysis in all 134 patient tumors in the trebananib arm and 132 of the 133 controls.

Activation of the Tie2 receptor as measured at two independent phosphorylation sites, S1119 and Y992, was positively associated with pCR in the trebananib-treated HER2+ cohort (Figure 4A, Supplementary Table S4) (p=0.001 and p=0.0007 respectively), but not the levels of the total Tie2 protein or mRNA. Moreover, downstream receptor pathway-linked AKT-mTOR protein signaling, such as phosphorylation of elF4G S1108 (p = 0.005), p70S6K T389 (p = 0.011), p70S6K T412 (p = 0.038) and FOXO3a S253 (p = 0.041), all associated with pCR, providing further biochemical evidence of the importance of functional Tie2 receptor activation for therapeutic response. In addition, Tie2 S1119, Tie2 Y992, elF4G S1108, ERBB2 Y877, and FOXO3a S253 all demonstrated significant treatment interaction (Supplementary Table S4).

Since activation of Tie2 receptor at two independent phosphorylation sites was associated with pCR, we assessed whether these two phosphoproteins were correlated. As shown in Figure 4b, most tumors with high Tie2 Y992 also had high Tie2 S1119. We defined thresholds to discriminate high/low expression for Tie2 S1119 and Y992 in the HER2+ population and assessed whether the combination of these markers could be used to identify patients likely to respond to trebananib (Figure 4B). When these expression thresholds were applied to the treated and control HER2+ populations, we observed an 83% pCR rate in HER2+ trebananib-treated patients vs. 37.5% for the corresponding controls (Figure 4C).

In the HR-negative/HER2-negative (triple negative) signature, where pCR rates were higher in the trebananib arm relative to control, mechanism-of-action biomarkers from the Ang/Tie2 pathway did not appear to predict response (Supplementary Table S4). Rather, lower relative levels of total Tie2 protein and phospho-ER S118 were associated with pCR in the treated triple-negative population vs controls, with neither demonstrating a significant treatment interaction. In an exploratory analysis, response in this cohort was associated with higher levels of immune related genes and signatures such as the CD8+ T-cell signature by Danaher and colleagues (25), as shown in Figure 4D, left (p=0.002) and not in controls (Figure 4D, right, p=0.241).

In the HR-positive/HER2-negative signature, Ang/Tie2 pathway biomarkers did not associate with response. Rather, higher levels of PI3K-AKT-mTOR pathway biomarkers, such as phospho-PI3K (p=0.05), AKT1 T308 (p=0.014) and AKT1 S473 (p=0.046), and endothelial adhesion markers ICAM1 (p=0.0021) and PECAM1 (p=0.0085) associated with pCR, as did lower levels of total estrogen receptor protein (p=0.047) (Supplementary Table S4).

DISCUSSION

Trebananib, an Ang-Tie2 neutralizing peptibody, was studied in the ongoing I-SPY2 adaptively randomized platform trial. It was combined with paclitaxel (+/− trastuzumab), followed by AC and surgical resection, compared to the control of paclitaxel (+/− trastuzumab) followed by AC. This was conducted in 10 biomarker signatures. The primary endpoint analyses (pCR) concluded that the trebananib arm did not meet the I-SPY2 pre-specified threshold for “graduation” (>85% predictive probability of success in a hypothetical phase III trial) overall or in any signature. However, there was drug activity in several signatures, with predictive probabilities that approached the threshold of phase III success (74%−79%) in HR-negative, HR-negative/HER2-positive, HR-negative/HER2-negative, and ultra-high MammaPrint risk (MP2) signatures. The arm reached maximum accrual, and a favorable safety profile was observed for this first-in-human neoadjuvant breast cancer trial of this novel agent.

Trebananib showed evidence of activity in several subtypes, as measured by comparisons of the arm’s pCR rates versus control in the prespecified signatures. The overall probability that the trebananib arm pCR rate was superior to that of the control arm was 99% for all signatures combined, 99% for the HR-negative, high MammaPrint risk MP2, and triple negative cohorts; and 93% for the HR-negative/HER2-positive group. The signatures listed

drive the overall superior rate. These very high probability rates provide support for further validation of these findings in signature-specific trials, given the relatively small samples sizes within each signature.

Studies in metastatic breast cancer had provided safety data for our trial, but did not report efficacy results until the current trial concluded. There was no significant improvement with trebananib (13,14). In the neoadjuvant setting, the I-SPY2 findings of favorable trebananib activity in selected subtypes and safety profile while very encouraging, were not acted upon with subsequent confirmatory trials. In large part this was because the company decided to end their trebananib program. However, these data provide important insights to the Tie2 pathway and other companies with novel agents targeting this pathway should find this information informative. Further study of this pathway is warranted. Importantly, with no major toxicities noted from trebananib, combination trials with other pathway-specific drug classes and/or immunotherapy could be designed in the neoadjuvant setting. Alternatively, larger trials in the signature subsets of highest activity could be conducted. Moreover, predictive biomarkers, if known, might be used to better select a population more likely to have a high response to this drug class. We chose this latter strategy as part of the translational exploratory component of this trial.

Given trebananib's activity, we conducted preplanned qualifying biomarker analyses based on its known mechanism of action targeting Tie2 receptor-ligand engagement to discover if there were significant predictors of trebananib response that might help better refine subsequent clinical evaluations and patient selection. Although small sample sizes precluded drawing definitive conclusions, we observed several notable trends. In the HER2-positive subgroup, increased baseline/pre-treatment levels of activation/phosphorylation of the Tie2 receptor itself (phosphorylated Tie2 Y1119 and Y992) and its downstream signaling partners, including AKT-mTOR (eIF4G S1108, ERBB2 Y877, and FOXO3a 5253) — all with significant treatment interaction p-values — may be if validated predictive of favorable treatment efficacy (increased pCR rate). Of note, these biomarker analyses did not correct for multiple hypothesis (comparisons) testing (see Methods); the p values provided are descriptive. Our goal was to better understand the biology underlying response to trebananib and to identify predictive markers that warrant validation in future studies of Ang/Tie2 inhibitors.

It is of interest that even though there was a 99% probability that pCR rates were superior to control in the triple negative cohort, unlike the HER2-positive signatures, this response was not associated with elevated Ang/Tie2 pathway signaling activity, thus a different biomarker is needed for prediction. This may be a reflection of the molecular heterogeneity and heterogeneous signaling drivers of triple-negative tumors compared to HER2-positive breast cancer. However, high expression of a CD8 T cell gene signature did associate with response in the triple negative cohort. This may reflect the importance of immune participation in treatment response in this subtype, as well as immune-modulating effects of trebananib (25–27). The RPPA-based protein signaling analysis was performed using LCM-enriched tumor epithelium as the cellular input for analysis. Previous work revealed the significant improvement in gene-protein-phosphoprotein concordances and overall accuracy of the protein/phosphoprotein data when upfront cellular enrichment was used (28–30).

Consequently, while proteomic analysis of the separately LCM-enriched stroma/immune populations are ongoing, any phosphoprotein/protein biomarker correlations with response arising from the stroma/immune ecology was not captured in this analysis here. There may be a rationale for combining inhibitors of the Ang/Tie2 axis with immunotherapy based on the ability of these agents to also induce immunogenic modulation that would lead to increased efficacy of this class of drugs (31).

A number of compounds are being evaluated in breast cancer that target the Ang/Tie2 axis with different mechanisms of action. These include angiopoietin-sequestering biologics such as MED13617 and LCO6, as well as selective small molecule inhibitors of the Tie2 kinase such as rebastinib (32). TMEM structures (Tumor Microenvironment of Metastasis), comprised of a high Tie2/high VEGF macrophage, an endothelial cell and a tumor cell, form microanatomical sites of mammary cancer cell intravasation and dissemination. TMEM "MetaSites" can be identified in human breast cancer and are associated with increased early metastatic disease (33). It is possible that these structures can facilitate metastatic spread during the course of neoadjuvant chemotherapy (34), thus, blockade of the Ang/Tie2 axis may enhance chemotherapy efficacy via this mechanism. Rebastinib blocks both recruitment and function of high Tie2 macrophages to the TMEM structure (32).

The biologic effects of Ang1 and Ang2 are context-dependent and can function as either a Tie2 agonist, partial agonist or antagonist (3). Thus, there is the potential that inhibiting this axis could also be harmful. One study reported that high Tie2 tumor cell expression is favorable and sufficient in inducing dormancy in vitro and in vivo and that it inhibits development of lytic bone metastases (35). In breast cancer trials to date with Ang/Tie2 inhibition, there has been no clinical evidence of/for worse outcome in the investigational arms. The data from the I-SPY2 arm suggest there may be a beneficial effect in some subtypes and agents targeting the Tie2 pathway could be combined with other compounds known to improve outcomes (HER2-targeted agents in the HER2 signatures, and PD-1 inhibitors in the TNBC signatures).

The clinical results reported here reinforce that the achievement of pCR in the neoadjuvant breast cancer setting is a robust predictor of improved EFS (36–39). Regardless of arm (trebananib or control), those with a pCR had a significantly improved and extremely favorable EFS despite their high-risk disease. Moreover, the EFS for trebananib vs control was superior overall and within several signatures, with hazard ratios ranging from 0.41–0.79, reflecting a 21%−59% improvement in outcome in the exploratory endpoint of EFS with the 12-week exposure of trebananib.

Our study has several important limitations and observations. The efficiency of I-SPY2's platform design and adaptive randomization approach leads to relatively small sample sizes that decrease the statistical power of the biomarker analyses. No formal statistical comparisons were conducted for the main EFS outcomes of trebananib vs control due to the randomized phase II design with pCR as the primary endpoint. This particularly was the case in the HER2-positive signatures due to the late entry of this group into the trebananib arm (because of need to wait for safety data of the trastuzumab-taxane combination in ongoing trials in the metastatic setting). While the results of the biomarker studies appear

promising, they could not be corrected for multiple comparisons and as such are hypothesis-generating and warranting validation, as discussed above. This study was not designed to test the optimal duration of neoadjuvant trebananib and whether it might be more efficacious if also added to the AC component. At least for the 12-week duration in the I-SPY2 trial, it was tolerated well.

One final observation is that the pCR rates observed are lower than expected, as reported in the I-SPY2 control arms of the majority of our legacy trials. We postulate that this in large part may be due to I-SPY2's use of standardized pathologic evaluation of surgical specimens by the RCB method to fully map gross and microscopic findings. The estimated pCR (RCB 0) rate reported in this manuscript for the triple-negative subset is in line with what we have observed in previous publications where we have observed estimated pCR rates ranging from 22%−31% (40,41). As for the HER2-positive subsets, our previous publications reported rates ranging from 17%−35%.(16,40) We recognize that the estimated pCR rate reported in this manuscript is lower in these subsets; however, the number of patients, particularly within the HR-negative/HER2-positive signature, is very small. As a result, the 95% probability interval (PI) for the estimated pCR rate is wide and the previously reported rates all fall within this range. Furthermore, paclitaxel/trastuzumab, not paclitaxel/trastuzumab/pertuzumab was the control standard used in these trials (prior to approval of pertuzumab in the neoadjuvant setting).

In summary, the Ang/Tie2 axis inhibitor trebananib, a neutralizing peptibody, when added to neoadjuvant paclitaxel +/− trastuzumab (versus the control of paclitaxel +/− trastuzumab) in the I-PY2 neoadjuvant trial for high risk, early-stage breast cancer was well tolerated in this phase II investigation. Although the arm did not meet I-SPY2's preset thresholds for phase III evaluation, trebananib showed major activity in this setting. The probability of trebananib's superiority in achieving a pCR versus control was 99% overall and greater than 93% in all clinical signatures except HR-positive subtypes. The predictive probability of success in a phase III trial ranged from 74% to 79% in the HR-negative, triple-negative, high MammaPrint risk MP2, and HR-negative/HER2-positive subtypes, for which the 3-year EFS was also numerically superior overall and in these signatures, compared to control. Qualifying biomarker analyses of multi-omic (gene-protein-phosphoprotein expression) Tie2 pathway biology based on trebananib's mechanism of action found that activation levels of the Tie2 receptor and its downstream signaling partners were associated with improved response in HER2-positive disease. Improved response in triple negative disease was associated with a high CD8 T cell gene signature. Collectively, our results provide rationale for future testing of new Ang/Tie2 receptor axis inhibitors in triple-negative, HR-negative and HR-negative/HER2-positive breast cancer (alone or in combination with other drug classes such as immunotherapy or anti-HER2 agents). These findings also support validation of pathway-specific biomarkers identified herein to select the optimal population for this novel approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations List:

VEGF	vascular endothelial growth factor
TEM	tie2-expressing macrophages
HR	hormone receptor
pCR	pathologic complete response
EFS	event-free survival
DRFS	distant recurrence-free survival
RCB	residual cancer burden
MP	MammaPrint
AC	doxorubicin (Adriamycin) and cyclophosphamide
NCCN	National Comprehensive Cancer Network
MRI	magnetic resonance imaging
LCM	laser capture microdissection
RPPA	reverse-phase protein array
KM	Kaplan-Meier
TMEM	tumor microenvironment of metastasis
TNBC	triple negative breast cancer

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TRANSLATIONAL RELEVANCE

The I-SPY2 phase II neoadjuvant adaptive platform trial evaluates experimental therapies in high-risk early-stage breast cancer. Tumors are randomized based on eight subtypes and evaluated within 10 clinical biomarker signatures (based on hormone receptor (HR), human epidermal growth factor receptor 2 (HER2), and high MammaPrint risk (MP1, MP2) statuses). The experimental agent is added to paclitaxel with/without trastuzumab, followed by doxorubicin/cyclophosphamide. The current study explored inhibition of the angiopoietin(Ang)Tie2 receptor axis, which plays a key role in tumor angiogenesis, by the Ang/Tie2 neutralizing peptibody trebananib. The drug was well-tolerated, and although trebananib reached maximum accrual without achieving predefined efficacy thresholds for phase III evaluation, several subtypes were close. There were higher rates of pCR relative to control in HR-negative, MP2, HR-negative/HER2-negative and HR-negative/HER2-positive signatures, supporting further study of Ang/Tie2-targeting agents. Pre-specified (e.g. qualifying) Tie2 receptor and pathway-specific biomarker analyses identified phosphorylated Tie2, but not total levels of Tie2, along with downstream pathway-linked signaling events as possible predictors of trebananib response in HER2-positive and HR-negative/HER2-negative signatures. Once validated, this suggests the potential utility of functional phosphoprotein-based biomarkers for patient selection/enrichment in future trials.

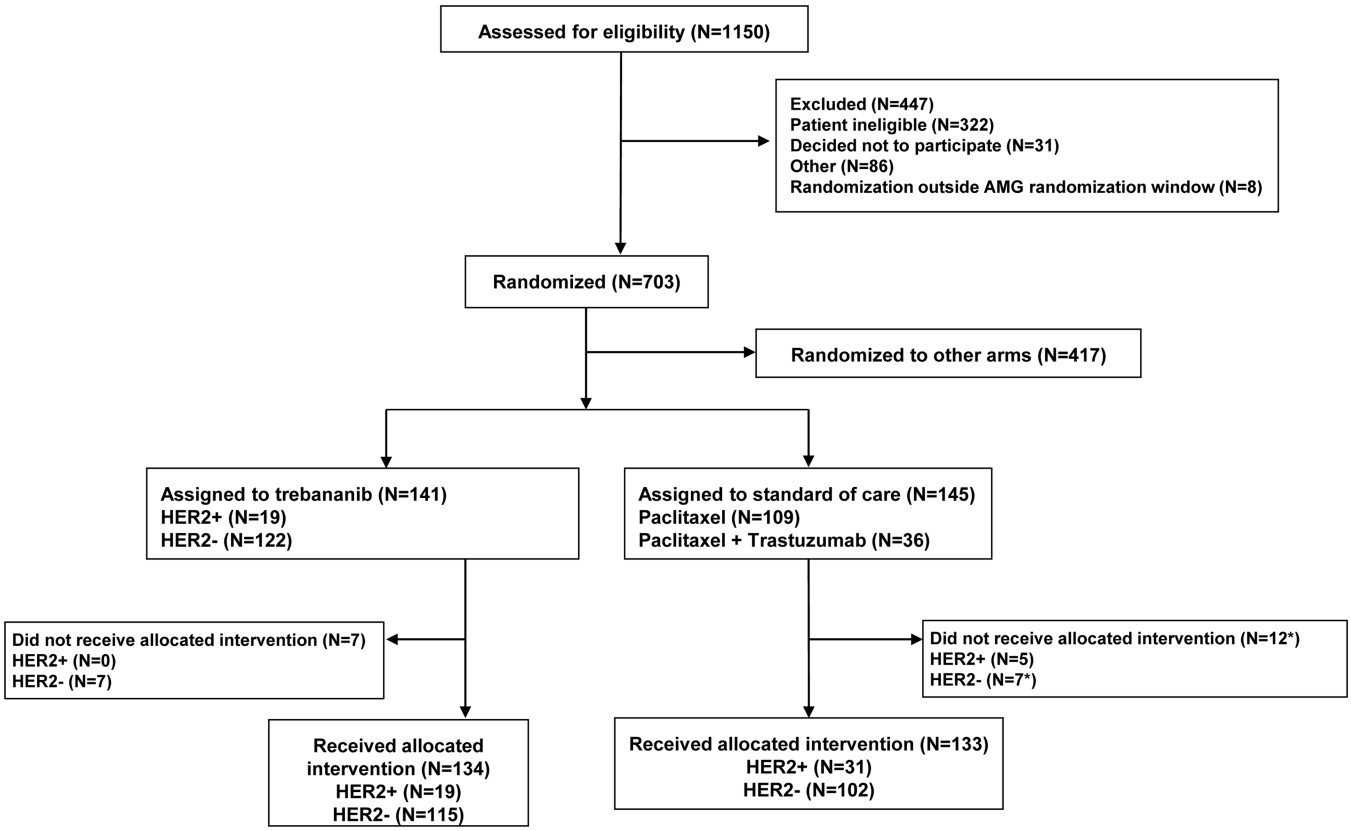

Figure 1.

CONSORT Diagram

* This includes one patient whose chemotherapy data was unknown at the time of graduation/final analysis

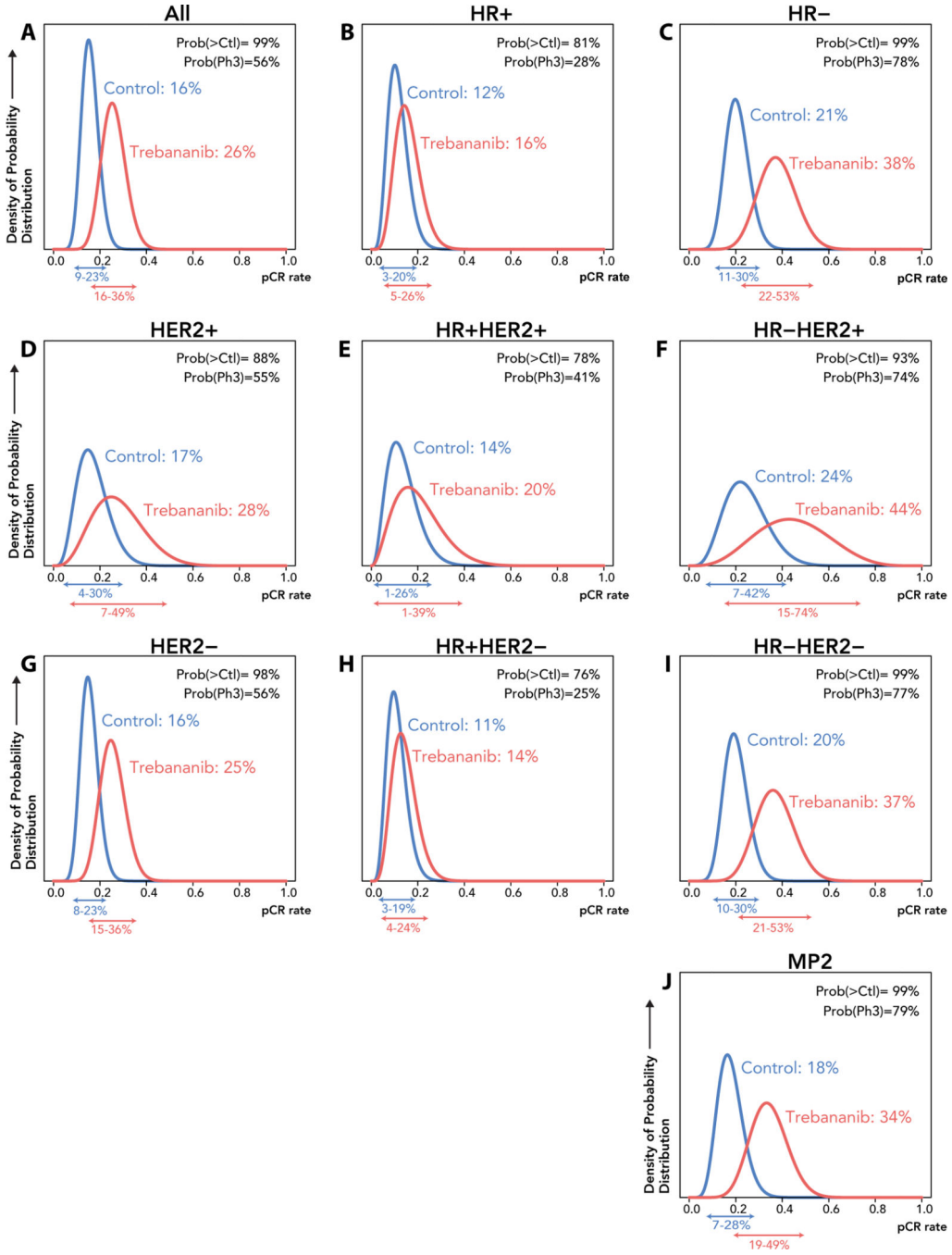

Figure 2.
Final pCR probability distributions

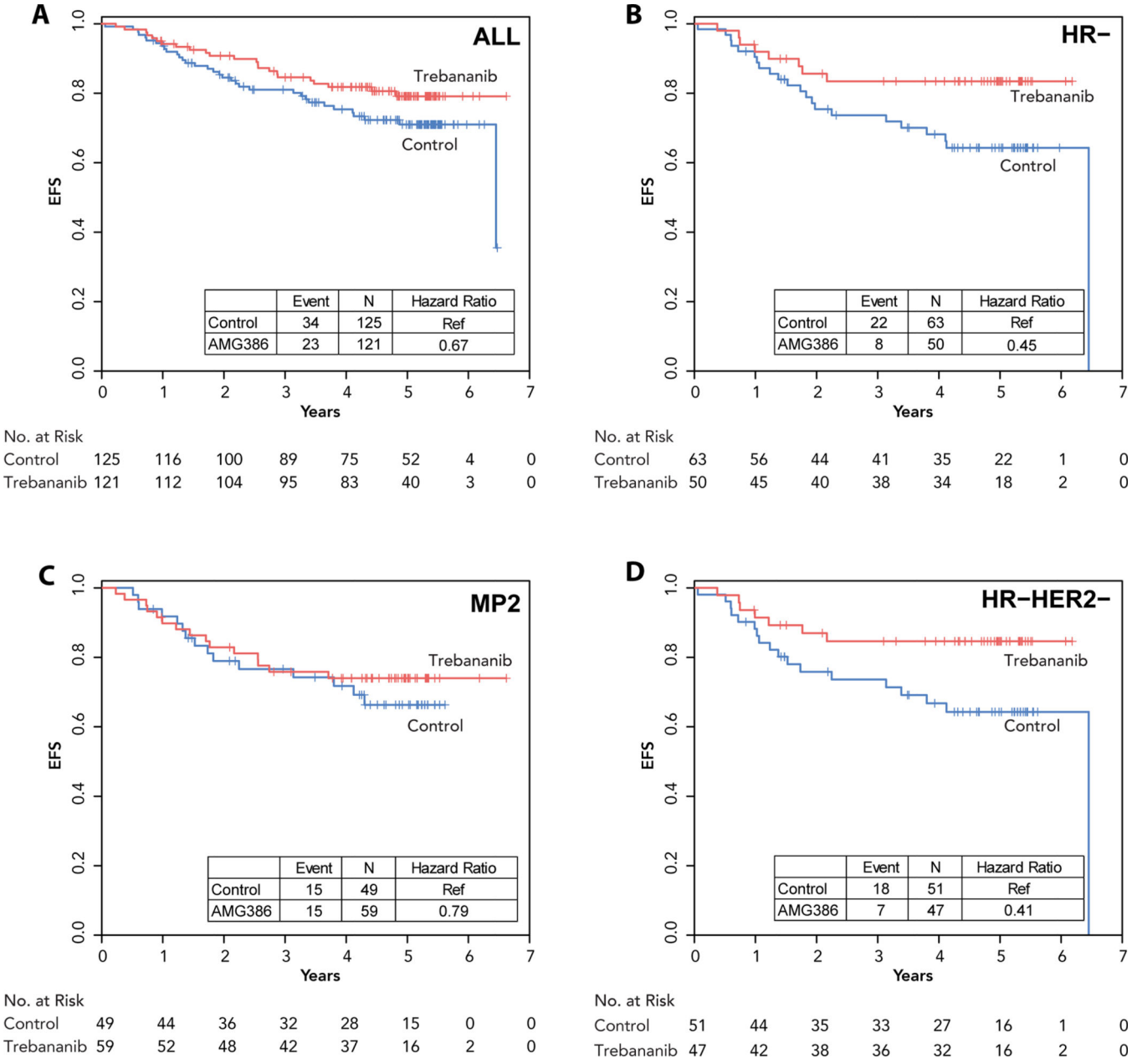

Figure 3.

Kaplan-Meier event-free survival plots of trebananib vs control arms overall and by selected signatures

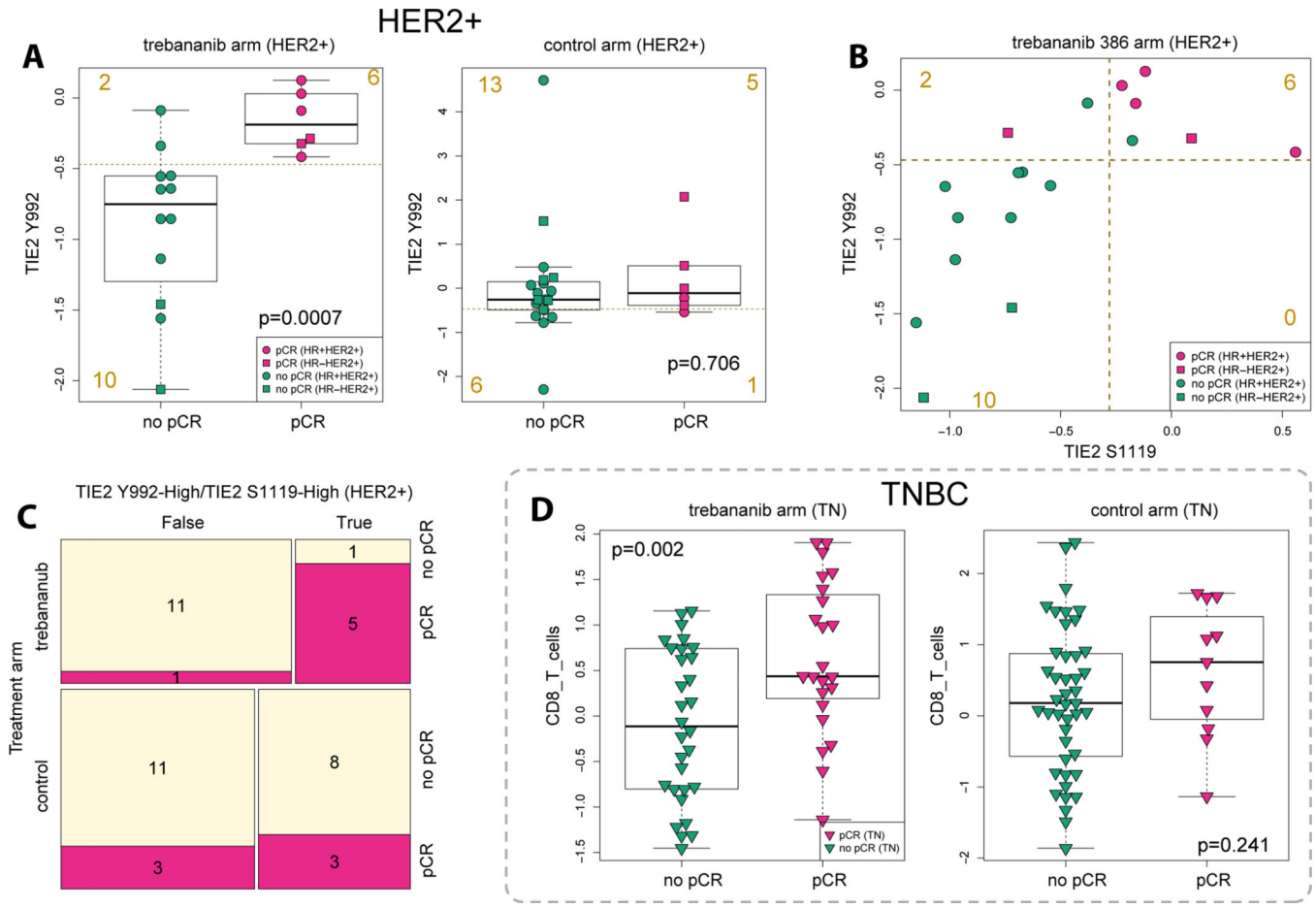

Figure 4:
Response-predictive biomarker studies

Table 1.

Baseline patient characteristics

Characteristic	Trebananib (n=134)	Control (n=133)
Median age (range), years	49 (25–68)	48 (24–71)
Race/ethnicity, n (%) White	109 (81%)	108 (81%)
African American	15 (11%)	16 (12%)
Asian	5 (4%)	8 (6%)
Other/Mixed	5 (4%)	1 (1%)
Hormone receptor status, n (%)		
Positive	77 (57%)	67 (50%)
Negative	57 (43%)	66 (50%)
HER2 status, n (%)		
Positive	19 (14%)	31 (24%)
Negative	115 (86%)	102 (76%)
MammaPrint high-risk status, n (%)		
MP1	71 (53%)	80 (60%)
MP2	63 (47%)	53 (40%)
Median tumor size by MRI (range), cm	3.5 (1.7–13.2)	3.7 (1.2–15.0)
Baseline nodal status, n (%)		
Palpable	50 (37%)	55 (41%)
Non-palpable	71 (53%)	72 (54%)
N/A	13 (10%)	6 (5%)

Table 2.

Grade 3/4 Adverse events occurring in 2% or greater of participants; dose reductions and early discontinuations (number, % of participants in arm/treatment phase).

	Trebananib (n=134)		Control (n=133)	
	Paclitaxel + trebananib (n=134)	**AC (n=108)**	**Paclitaxel (n=133)**	**AC (n=111)**
Adverse Events				
Anemia	0 (0.0%)	10 (9.3%)	1 (0.8%)	5 (4.5%)
Febrile neutropenia	1 (0.7%)	6 (5.6%)	1 (0.8%)	10 (9.0%)
Diarrhea	2 (1.5%)	1 (0.9%)	3 (2.3%)	1 (0.9%)
Stomatitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (2.7%)
Alanine aminotransferase increase	4 (3.0%)	0 (0.0%)	3 (2.3%)	0 (0.0%)
Neutropenia	9 (6.7%)	11 (10.2%)	6 (4.5%)	6 (5.4%)
Leukopenia	5 (3.7%)	8 (7.4%)	4 (3.0%)	5 (4.5%)
Hypokalemia	1 (0.7%)	4 (3.7%)	1 (0.8%)	3 (2.7%)
Hypertension	5 (3.7%)	1 (0.9%)	7 (5.3%)	4 (3.6%)
Treatment Delivery				
Dose Reductions, n (%)	7 (5.2%)	6 (5.6%)	2 (1.5%)	7 (6.3%)
Early Discontinuation, n (%)				
All reasons	26 (19.4%)	5 (4.6%)	22 (16.5%)	7 (6.3%)
Toxicity	10 (7.5%)	2 (1.9%)	5 (3.8%)	4 (3.6%)
Progression	9 (6.7%)	0 (0.0%)	8 (6.0%)	1 (0.9%)
Other	7 (5.2%)	3 (2.8%)	9 (6.8%)	2 (1.8%)
Time from Treatment Consent to Surgery (days)				
Median (range)	172 (97 – 265)		170 (100 – 289)	