8-2-2022

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ABSTRACT

Purpose: To evaluate prediction of response and event-free survival (EFS) following neoadjuvant endocrine therapy by SET2,3 index of nonproliferation gene expression related to estrogen and progesterone receptors adjusted for baseline prognosis.

Experimental Design: A correlative study was conducted of SET2,3 measured from gene expression profiles of diagnostic tumor (Agilent microarrays) in 379 women with cStage II–III breast cancer from the American College of Surgeons Oncology Group Z1031 neoadjuvant aromatase inhibitor trial SET2,3 was dichotomized using the previously published cutoff. Fisher exact test was used to assess the association between SET2,3 and low proliferation at week 2–4 [Ki67 ≤ 10% or complete cell-cycle arrest (CCCA; Ki67 ≤ 2.7%)] and PEPI-0 rate in cohort B, and the association between SET2,3 and ypStage 0/I in all patients. Cox models were used to assess EFS with respect to SET2,3 excluding cohort B patients who switched to chemotherapy.

Results: Patients with high SET2,3 had higher rate of pharmacodynamic response than patients with low SET2,3 (Ki67 ≤ 10% in 88.2% vs. 56.9%, $P < 0.0001$; CCCA in 50.0% vs. 26.2%, $P = 0.0054$), but rate of ypStage 0/I (24.0% vs. 20.4%, $P = 0.4580$) or PEPI = 0 (28.4% vs. 20.6%, $P = 0.3419$) was not different. Patients with high SET2,3 had longer EFS than patients with low SET2,3 (HR, 0.52, 95% confidence interval: 0.34–0.80; $P = 0.0026$).

Conclusions: This exploratory analysis of Z1031 data demonstrated a higher rate of pharmacodynamic suppression of proliferation and longer EFS in high SET2,3 disease relative to low SET2,3 disease. The ypStage 0/I rate and PEPI = 0 rate were similar with respect to SET2,3.

Introduction

Neoadjuvant endocrine therapy (NeoET) trials for patients with clinical stage II–III breast cancer that is hormone receptor–positive and HER2-negative ($HR^+/HER2^-$) provide an opportunity to evaluate the impact of a relatively short preoperative exposure to endocrine therapy on the cellular and/or pathologic response in the primary tumor, yet retain the ability to offer chemotherapy later if deemed appropriate (1–3). However, a means is needed to identify which patients presenting with clinical stage II–III $HR^+/HER2^-$ disease are most appropriate to begin NeoET. The American College of Surgeons Oncology Group (ACOSOG) Z1031 trial is one of few prospective clinical trials that is sufficiently powered for evaluation of response and long-term patient outcomes after NeoET for clinical stage II–III $HR^+/HER2^-$ breast cancer (4, 5).

The SET2,3 assay of sensitivity to endocrine therapy genomic index for stage II–III breast cancer was designed to measure nonproliferative hormone receptor–related transcription and then adjust for the patient's baseline prognosis from disease burden (tumor size and regional lymph node involvement) combined with molecular subtype (6–8). These two components of SET2,3 are the $SET_{ER/PR}$ index (18 nonproliferative hormone receptor–related transcripts relative to 10 reference transcripts) and the baseline prognostic index (BPI) that combines clinical tumor stage, clinical nodal (cN) stage, and RNA4 (molecular subtype derived from four transcripts: *ESR1*, *PGR*, *ERBB2*, and *AURKA*) measured from the pretreatment tumor biopsy (7, 8). Higher $SET_{ER/PR}$ index values indicate more active endocrine-related transcription and higher BPI values indicate more indolent prognostic features (7). $SET_{ER/PR}$ and BPI have been reported to add independent prognostic information in the setting of adjuvant chemoendocrine therapy for $HR^+/HER2^-$ breast cancer (7). SET2,3 represents their weighted sum ($SET2,3 = 0.75 \times SET_{ER/PR} + 0.51 \times BPI$; refs. 7, 9).

The objective of this study was to examine whether SET2,3 could identify patients who are more likely to experience tumoral response to NeoET, including early on-treatment pharmacodynamic response, posttreatment pathologic response, or improved long-term clinical outcomes.

Materials and Methods

Study cohort

The patients included in this study were drawn from the ACOSOG neoadjuvant trial Z1031, a phase III trial that enrolled postmenopausal

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

ClinicalTrials.gov Identifier: NCT00265759.

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Clin Cancer Res 2022;28:3287–95

doi: 10.1158/1078-0432.CCR-22-0068

Translational Relevance

Trials of neoadjuvant endocrine therapy (NeoET) in cStage II–III breast cancer have established that early pharmacodynamic and subsequent pathologic response to NeoET are prognostic, so some patients may avoid chemotherapy. But there is still a need for pretreatment biomarkers to identify the most appropriate patients to begin NeoET. In this blinded analysis of the sensitivity to endocrine therapy (SET2,3) index in the American College of Surgeons Oncology Group Z1031 trial of NeoET, we demonstrate that higher SET2,3 from diagnostic tumor biopsy was associated with greater odds of early pharmacodynamic response in the tumor (Ki67 ≤ 10% after 2–4 weeks NeoET) and longer event-free survival (EFS). Individual components of SET2,3, measuring endocrine receptor–related transcription ($SET_{ER/PR}$) and baseline prognosis were found to be independently prognostic for EFS.

women with clinical stage II–III estrogen receptor (ER)–positive Allred score 6–8 (or ER-positive staining in >66.7% cells) breast cancer 4)). ACOSOG is now part of the Alliance for Clinical Trials in Oncology. Z1031 was conducted in two sequential cohorts. Patients in cohort A were randomized to 16 to 18 weeks of either 1 mg anastrozole daily, 2.5 mg letrozole daily, or 25 mg exemestane daily (4). Patients in cohort B chose to receive either anastrozole or letrozole and underwent a tumor biopsy after 2 to 4 weeks of treatment. If the tumor had Ki67 > 10% at week 2–4, it was recommended that they switch to chemotherapy (cohort B-chemo) or go immediately surgery (cohort B-surgery; ref. 10). Otherwise, if the tumor had Ki67 ≤ 10% at week 2–4 or there were insufficient tumor cells in the specimen to quantify proliferation, it was recommended that patients remain on their endocrine treatment (cohort B-endo). Patients enrolled onto cohorts A and B had tumor specimens collected prior to start of neoadjuvant endocrine treatment and at surgery, for research purposes. cohort B also had a research tumor specimen collected after 2 to 4 weeks of treatment. These specimens were sent to Washington University, St. Louis, for storage, central immunostaining (Ki67) and gene expression microarrays, as per the clinical trial protocol for ACOSOG Z1031 (NCT00265759, Clinical Trials Support Unit, NCI; ref. 4). Participants in ACOSOG Z1031 signed an Institutional Review Board (IRB)–approved, protocol-specific written informed consent document for use of their data and samples, in accordance with institutional and federal guidelines (U.S. Common Rule). In addition, the central data analysis reported in this study was approved with waiver of consent (not human subjects research) by the IRB at MD Anderson Cancer Center (protocol LAB04-0093) and the Alliance for Clinical Trials in Oncology (approval A151701).

Data collection and statistical analyses were conducted by the Alliance Statistics and Data Management Center. Data quality was ensured by review of data by the Alliance Statistics and Data Management Center and by the principal investigator following Alliance policies.

RNA processing and SET determination

We analyzed the expression levels of 31 transcripts from pre-existing gene expression data from Agilent 4×44K Whole Human Genome Microarrays that were processed from frozen tumor biopsy cores using methods described previously (4). The Z1031 investigators provided gene expression data for the 31 transcripts used in the SET2,3 algorithm to the non-ACOSOG bioinformatician (L. Du) in Dr. Symmans's laboratory in 2018, who was blinded to patient treatment and clinical outcomes. SET2,3 index and its components, $SET_{ER/PR}$ and BPI, were calculated and provided to the ACOSOG trial statistician (V.J. Suman) to interrogate their association with clinical outcome under an Alliance for Clinical Trials data sharing agreement (A151701). All microarray data from Z1031 were subsequently made publicly available in the Gene Expression Omnibus (GEO; ref. 11).

Statistical analysis plan

SET2,3 was dichotomized using the cut-off point derived from the chemoendocrine treatment setting (namely, 1.77; ref. 7). Its components, $SET_{ER/PR}$ and BPI, were dichotomized using their median value (namely, 1.36 and 1.5, respectively) among the 379 women of cohorts A and B.

Endpoints of this study included two biomarkers of early pharmacodynamic response, defined as the proportion of patients whose week 2–4 Ki67 ≤ 10% and the proportion of patients whose tumor was in complete cell-cycle arrest (CCCA), that is Ki67 ≤ 2.7% at week 2–4 of NeoET. Because prior to neoadjuvant endocrine treatment (pre-NeoET) Ki67 differed between patients with low versus high SET2,3, logistic regression was used to assess the effect of SET2,3 on week 2–4 Ki67 outcomes adjusted for pre-NeoET Ki67 level. Two additional endpoints of pathologic response at time of surgery were the proportion of patients with preoperative endocrine therapy prognostic index group zero (PEPI = 0) and the proportion of patients with ypStage0/I disease. Stage was evaluated in both cohorts and PEPI = 0 rate was evaluated in cohort B only (where it was a predefined endpoint). PEPI = 0 rate is defined as the number of patients with a pathologic complete response (pCR) or ypT1–2 ypN0, ER-positive IHC (Allred score: 3–8), and Ki67 ≤ 2.7% in the residual invasive cancer, and was assessed among all the women in cohort B who began NeoET (12). Women in cohort B who either had a week 2–4 Ki67 > 10% or failed to undergo surgery after 12 weeks NeoET were considered to have a nonzero PEPI score and not to have ypStage0/I disease. Fisher exact test was used to assess whether biomarkers of response differed with respect to SET2,3 and its components.

The clinical outcome of interest was event-free survival (EFS) postregistration, defined as time from registration to local, regional, or distant disease progression/recurrence, second invasive primary cancer, or death due to any cause. Those who died without documented disease progression were censored at their last disease evaluation. Disease progression occurring during the neoadjuvant period was confirmed by imaging. Cox modeling was used to evaluate whether EFS differed with respect to the SET2,3 or its components among the women of cohort A and cohort B-endo. Because BPI is a discrete variable (possible values in increments of 0.5), it was dichotomized at median, whereas $SET_{ER/PR}$ was treated as a continuous variable in the Cox model. We used threshold plots to represent the odds of response (week 2–4 Ki67 ≤ 10%, CCCA, PEPI = 0) for SET2,3 index values at or above a given threshold relative to the odds for SET2,3 index below that threshold, plotting the threshold values from the 25th to 75th percentile of the SET2,3 index distribution. A similar approach was used for EFS with threshold plots to represent the hazard of EFS event for SET2,3 index values at or below each threshold relative to the hazard above that threshold. All analyses were based on the study database frozen on June 5, 2019 (5).

Data availability

The data generated in this study are publicly available in GEO at GSE87411 and GSE13644 (11).

Results

Disease characteristics prior to neoadjuvant endocrine treatment

SET2,3 could be ascertained from the baseline (pre-NeoET) tumor biopsy for 379 women (**Fig. 1**; **Table 1**). Approximately 15% of these women self-reported Black or African American race or other (**Table 1**). Supplementary Table S1 indicates that differences seen between those with RNA profiling results and those without include ascertainment of pre-NeoET Ki67 (cohorts A and B), tumor grade (cohorts A and B); cN stage (cohort B), Eastern Cooperative Oncology Group (ECOG) performance score (cohort B), and triage to neoadjuvant chemotherapy (NAC; cohort B). There were 166 (45.7%) patients known to have a pre-NeoET Ki67 result <15%. Moreover, 162 (42.7%) women who presented with clinical stage II disease and pre-NeoET Ki67 ≥ 15%, 160 (42.2%) women who presented with clinical stage II disease and Ki67 < 15% or unknown, 35 (9.2%) women who presented with clinical stage III disease and Ki67 ≥ 15%, and 22 (5.8%) women who presented with clinical stage III disease and Ki67 < 15% or unknown.

The distributions of SET2,3 and its component indices in the tumor biopsy prior to NeoET were as follows: SET2,3 median 1.74, interquartile range (IQR) 1.35–2.13; $SET_{ER/PR}$ median 1.36, IQR, 0.86–1.85; and BPI median 1.5, IQR, 1.0–1.5. High SET2,3 (>1.77) was present in 48.3% of patients (183/379) and was associated with older age (median 66 vs. 63 years, $P = 0.0164$) and cStage II versus III (95.1% vs. 75.5%, $P < 0.0001$). Among 183 (48.3%) women with high SET2,3 index prior to NeoET, 117 had high levels of both its components and 66 had only one component with high level. Among 196 women with low SET2,3 index prior to NeoET, 66 had low levels of both its components and 130 had only one component with a low level.

The proportion of women whose tumor had Ki67 ≥ 15% prior to NeoET was significantly greater ($P < 0.0001$) among those with low SET2,3 index (67.0%) relative to those with high SET2,3 index (40.6%) prior to NeoET, and a similar association was observed for the component indices $SET_{ER/PR}$ and BPI (**Table 2**).

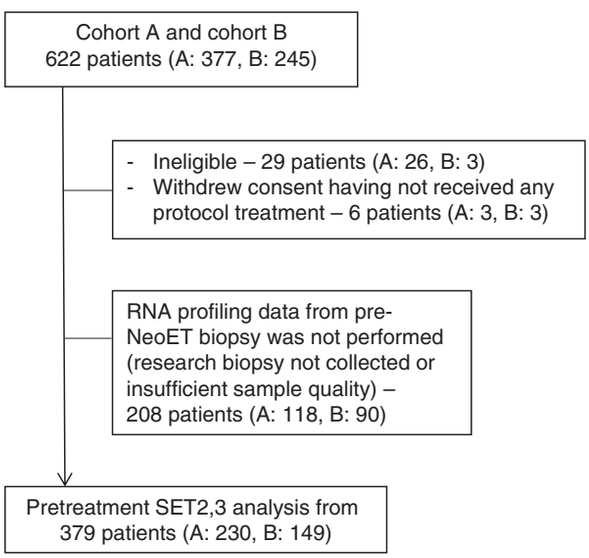

Figure 1.
REMARK diagram describing the cohort for analysis of SET2,3 index measured from the pretreatment tumor biopsy.

Table 1. Characteristics of the study population ($N = 379$).

Patient and disease characteristics	Study population No. (%)
Cohort	
A	230 (60.7%)
B-endocrine	114 (30.1%)
B-chemotherapy	29 (7.7%)
B-surgery	6 (1.6%)
Race	
Black/African American	51 (13.5%)
White	321 (84.7%)
Other	7 (1.8%)
Age at trial entry	
Less than 50 years old	3 (0.8%)
50–69 years old	249 (65.7%)
70 years of age or older	127 (33.5%)
ECOG performance status	
1	297 (78.4%)
2	72 (19.0%)
3	10 (2.6%)
Clinical stage	
II	322 (85.0%)
III	57 (15.0%)
Tumor grade	
1	82 (21.6%)
2	233 (61.5%)
3	63 (16.6%)
Not reported	1 (0.3%)
Central laboratory findings	
Preneoadjuvant treatment Ki67	
<15.0%	166 (43.8%)
≥15.0%	197 (52.0%)
Unknown	16 (4.2%)
Preneoadjuvant treatment SET2,3 index	
Low	196 (51.7%)
High	183 (48.3%)
Pre-RNA4_risk	
Low	30 (7.9%)
Borderline	87 (23.0%)
High	262 (69.1%)
Pre-*ESR1* positive (RNA)	376 (99.2%)
Pre-*ERBB2* positive (RNA)	3 (0.8%)

Biomarkers of early pharmacodynamic response to endocrine treatment

Among the 149 women in cohort B, 141 had Ki67 measured at 2 to 4 weeks, with Ki67 ≤ 10% in 104 (73.7%), including 72 who had pretreatment Ki67 > 10%, 31 whose pretreatment and week 2–4 Ki67 were both ≤10%, and one patient who was missing pretreatment Ki67, and CCCA (Ki67 ≤ 2.7%) in 55 (39.0%), including 50 who had pretreatment Ki67 > 2.7%, 4 whose pretreatment and week 2–4 Ki67 were both ≤2.7%, and one patient who was missing pretreatment Ki67. The median SET2,3 index prior to NeoET was significantly higher in the 104 women who had Ki67 ≤ 10% at 2 to 4 weeks, compared with 37 who had Ki67 > 10% ($P < 0.0001$). Also, the likelihood of Ki67 ≤ 10% after 2 to 4 weeks of NeoET was significantly greater ($P < 0.0001$) among those with high SET2,3 prior to NeoET (88.2%) than those with low SET2,3 (56.9%). Similar trends were observed for the component indices $SET_{ER/PR}$ and BPI (**Table 2**). The association between high SET2,3 and Ki67 ≤ 10%

Table 2. Associations of SET2,3 and its component indices ($SET_{ER/PR}$ and BPI) with measures of response to treatment.

Cohorts A and B	Pre-NeoET SET2,3 index low ($n = 196$)	Pre-NeoET SET2,3 index high ($n = 183$)	Pre-NeoET $SET_{ER,PR}$ index low ($n = 190$)	Pre-NeoET $SET_{ER,PR}$ index high ($n = 189$)	Pre-NeoET BPI low ($n = 138$)	Pre-NeoET BPI high ($n = 241$)
Pre-NeoET Ki67						
<15.0%	62 (33.0%)	104 (59.4%)	68 (37.4%)	98 (54.1%)	42 (31.6%)	124 (53.9%)
≥15.0%	126 (67.0%)	71 (40.6%)	114 (62.6%)	83 (45.9%)	91 (68.4%)	106 (46.1%)
(unknown)	(8)	(8)	(8)	(8)	(5)	(11)
	$P < 0.0001$		$P = 0.0016$		$P < 0.0001$	
ypT0-I ypN0 residual disease						
Yes	40 (20.4%)	44 (24.0%)	45 (23.7%)	39 (20.6%)	12 (8.7%)	72 (29.9%)
No	156 (79.6%)	139 (76.0%)	145 (76.3%)	150 (79.4%)	126 (91.3%)	169 (70.1%)
	$P = 0.4580$		$P = 0.5367$		$P < 0.0001$	

Cohort B	Pre-NeoET SET2,3 index low ($n = 68$)	Pre-NeoET SET2,3 index high ($n = 81$)	Pre-NeoET $SET_{ER,PR}$ index low ($n = 71$)	Pre-NeoET $SET_{ER,PR}$ index high ($n = 78$)	Pre-NeoET BPI low ($n = 56$)	Pre-NeoET BPI high ($n = 93$)
Complete cell-cycle arrest						
Week2–4 Ki67 ≤2.7%	17 (26.2%)	38 (50.0%)	21 (31.8%)	34 (45.3%)	12 (21.8%)	43 (50.0%)
Week2–4 Ki67 >2.7%	48 (73.8%)	38 (50.0%)	45 (68.2%)	41 (54.7%)	43 (78.2%)	43 (50.0%)
(unknown)	(3)	(5)	(5)	(3)	(1)	(7)
	$P = 0.0054$		$P = 0.1205$		$P = 0.0008$	
Week2–4 Ki67 ≤10%	37 (56.9%)	67 (88.2%)	43 (65.2%)	61 (81.3%)	34 (61.8%)	70 (81.4%)
Week2–4 Ki67 >10%	28 (43.1%)	9 (11.8%)	23 (34.8%)	14 (18.7%)	21 (38.2%)	16 (18.6%)
(unknown)	(3)	(5)	(5)	(3)	(1)	(7)
	$P < 0.0001$		$P = 0.0355$		$P = 0.0116$	
PEPI = 0						
Yes	14 (20.6%)	23 (28.4%)	18 (25.4%)	19 (24.4%)	6 (10.7%)	31 (33.3%)
No	54 (79.4%)	58 (71.6%)	53 (74.6%)	59 (75.6%)	50 (89.3%)	62 (66.7%)
	$P = 0.3419$		$P = 0.9999$		$P = 0.0018$	

Abbreviations: BPI, baseline prognostic index; PEPI = 0, preoperative endocrine therapy prognostic index group zero; Week2–4 Ki67, percent tumor cells expressing Ki67 after 2–4 weeks of NeoET.

after 2 to 4 weeks remained statistically significant after adjusting for whether pretreatment Ki67 was ≤10% or not, with adjusted OR for high SET2,3 relative to low SET2,3 of 5.18 [95% confidence interval (CI): 2.19–12.26, $P = 0.0002$]. Moreover, we observed that the estimated odds of Ki67 ≤ 10% after 2 to 4 weeks of NeoET were increased (significantly above 1.0) for those with SET2,3 that was above, compared with below, any threshold within the IQR of SET2,3 (**Fig. 2A**).

The median SET2,3 index prior to NeoET was higher in the 55 (39.0%) women who had CCCA (Ki67 ≤ 2.7%) in the week 2–4 tumor biopsy, compared with 86 who had Ki67 > 2.7% ($P = 0.0023$). The proportion of women with tumor in CCCA after 2–4 weeks of NeoET was found to be significantly higher ($P = 0.0030$) among those with high SET2,3 index prior to NeoET (50.0%) than those with low SET2,3 index (24.6%). A similar trend was observed for the component indices $SET_{ER/PR}$ and BPI (**Table 2**). The association between high SET2,3 and CCCA remained statistically significant after adjusting for whether pretreatment Ki67 ≤ 2.7% or not, with adjusted OR 2.78 (95% CI: 1.35–5.69, $P = 0.0053$) for high SET2,3 relative to low SET2,3. Moreover, we observed that the estimated odds of CCCA after 2 to 4 weeks of NeoET was increased for those with SET2,3 that was above, compared with below, any threshold within the IQR of SET2,3 (**Fig. 2B**).

Pathologic response

Among the 379 women of cohorts A and B, 84 (22.2%) remained on NeoET and had ypStage 0–I at time of surgery. Median pre-NeoET SET2,3 index was significantly higher among those with ypStage 0–I versus those with ypStage II–III ($P = 0.0197$), but the proportion of women with ypStage 0–I disease after NeoET was not found to differ significantly according to whether pre-NeoET SET2,3 index was high or low, or whether $SET_{ER/PR}$ index was high or low (**Table 2**). However, a higher proportion of women with a high pre-NeoET BPI had ypStage0–I disease after NeoET than that of women with a low BPI (29.9% vs. 8.7%: $P < 0.0001$).

Among the 149 women that comprise cohort B, 37 (24.8%) of the women remained on NeoET and had PEPI = 0 response at time of surgery. Their baseline tumor samples tended to have higher median SET2,3 prior to NeoET compared with the 112 that had PEPI ≥ 1 after 16–18 weeks of NeoET (SET2,3 index median 1.93 vs. 1.82, $P = 0.0895$). The proportion of patients with PEPI = 0 after NeoET was not found to differ significantly with respect to whether SET2,3 index prior to NeoET was high or low (**Table 2**). Moreover, the likelihood of a PEPI = 0 response was not found to differ among those with high SET2,3 prior to NeoET and those with low SET2,3 prior to NeoET for any threshold value in the IQR (**Fig. 2C**). In terms of the SET2,3 components, the proportion of patients with PEPI = 0 after NeoET was not found to differ significantly with respect to

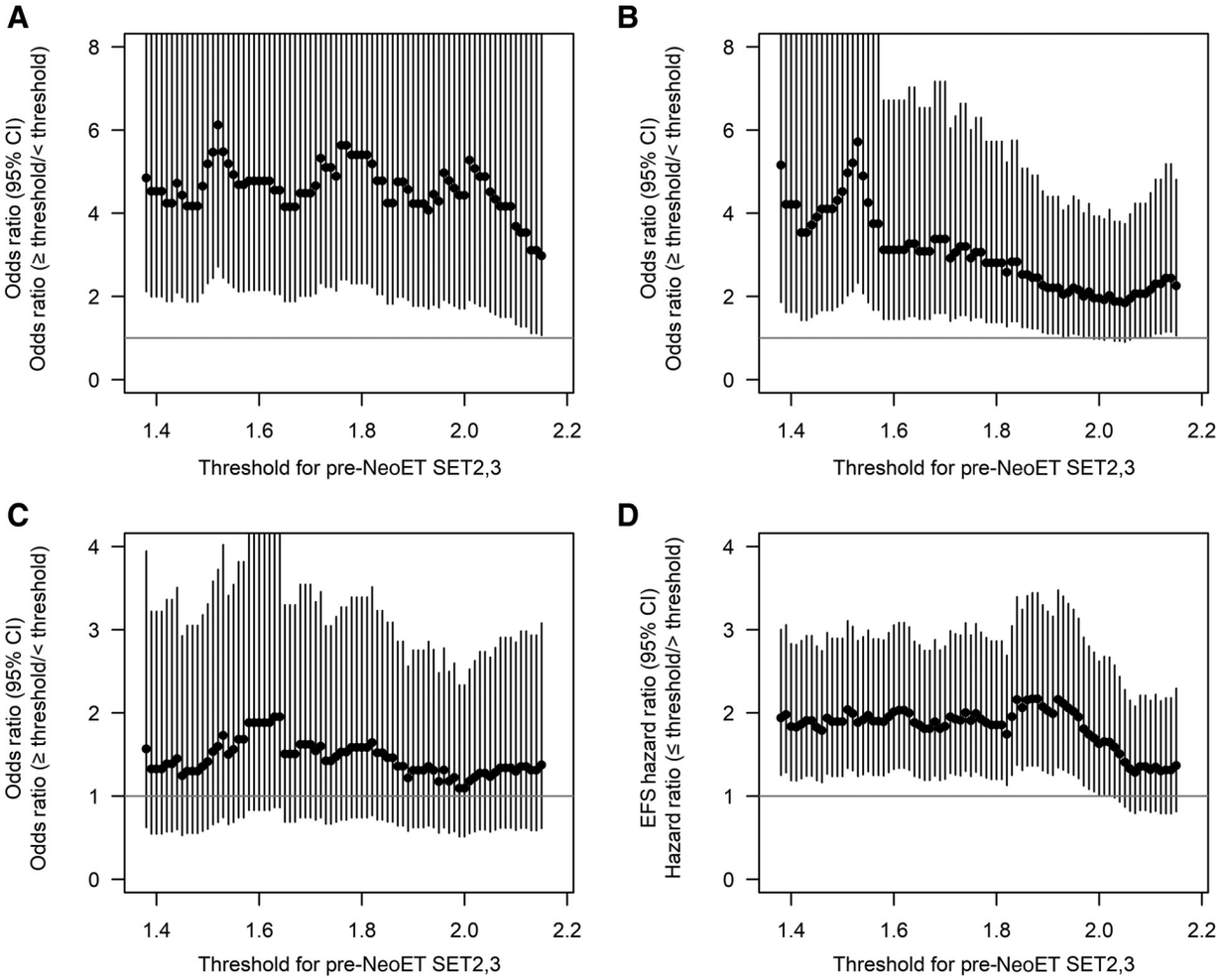

Figure 2.

Threshold plots for continuous SET2,3 index values in a tumor before treatment begins and the odds of a response from NeoET (in **A–C**) or the relative hazard of an EFS event (in **D**), as follows: tumoral Ki67 ≤ 10% at 2–4 weeks (**A**), complete cell-cycle arrest (tumoral Ki67 ≤ 2.7%) at 2–4 weeks (**B**), PEPI = 0 at surgery after 16–18 weeks of NeoET (**C**), and EFS (**D**). The relative odds (**A–C**) or relative hazard (**D**) and corresponding 95% CI are shown for values above, versus at or below, each value of SET2,3 in the IQR from 25th to 75th percentile.

whether pre-NeoET $SET_{ER/PR}$ was high or low, but a higher proportion of women with a high pre-NeoET BPI had PEPI = 0 score after NeoET than that of women with a low BPI (high: 33.3% vs. 10.7%: $P = 0.0018$).

Patient outcomes

Among the 344 women who comprise cohort A and cohort B-endo, there were 88 events related to EFS observed during follow-up, including 56 among patients with low SET2,3 index prior to NeoET and 32 among patients with high SET2,3 index value prior to NeoET. The median time to event after start of NeoET was 3.2 years (range: 1 month to 9 years), while the median length of follow-up after start of NeoET among those censored at last breast evaluation without an event was 7.5 years (range: 1 month to 9 years). We observed 44 events of disease progression (28 distant, 16 locoregional) and 12 second primary cancers (including non-breast primaries) among the 170 patients with a low SET2,3 index value prior to NeoET and 17 events of disease progression (12 distant, 5 locoregional) and 15 second primary cancers (including non-breast primaries) among the 174 patients with a high SET2,3 index value prior to NeoET. Thresholds of SET2,3 index prior to NeoET below 2.0 had significant HR in an exploratory analysis (**Fig. 2D**). EFS was significantly decreased among those with low SET2,3 index relative to those with high SET2,3 index value, using the predefined cutoff (**Fig. 3A**; HR, 0.52; 95% CI: 0.34–0.80 for high SET2,3 relative to low SET2,3; log-rank $P = 0.0026$). A similar result was observed when adjusted for pretreatment Ki67 (≥15% vs. >15%) with adjusted HR, 0.51 (95% CI: 0.29–0.90, $P = 0.0205$) for high relative to low SET2,3 at the predefined cutoff.

High BPI (dichotomized at median, HR, 0.47; 95% CI: 0.31–0.72; $P = 0.0005$) and higher $SET_{ER/PR}$ index (continuous variable, HR, 0.62; 95% CI: 0.45–0.87; $P = 0.0051$) were independently prognostic for EFS (**Table 3**). $SET_{ER/PR}$ index at or above median value indicated an improved prognosis in higher-risk tumors (BPI below median), whereas little difference was observed in the lower-risk tumors with BPI at or above median (**Fig. 3B**). Conversely, larger prognostic difference between BPI groups (low vs. high) was observed when

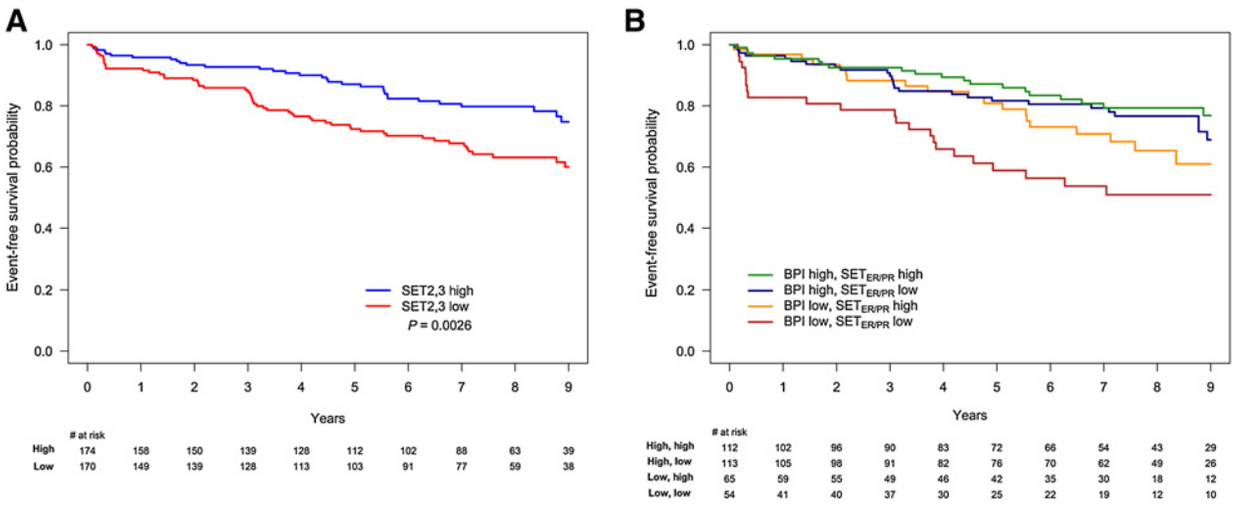

Figure 3.

Kaplan–Meier plots of EFS in the patients who completed NeoET by: pretreatment SET2,3 index defined as high or low using the predefined cut-off point (**A**); and the component indices BPI and $SET_{ER/PR}$ as dichotomous variables, categorized as high versus low based on whether the value was at or above the median versus below (**B**).

$SET_{ER/PR}$ index was below median value (**Fig. 3B**). When both component indices were median dichotomized in a multivariate Cox model, BPI was significant (HR, 0.49; 95% CI: 0.32–0.74; $P = 0.0008$) and $SET_{ER/PR}$ index had near-significant trend (HR, 0.68; 95% CI: 0.44–1.03; $P = 0.07$).

Discussion

The SET2,3 genomic signature for sensitivity to endocrine therapy provides a measurement of nonproliferative endocrine-related transcription that is adjusted for baseline prognosis (7). Using the published cut-off point for prognosis after chemoendocrine therapy (7), approximately 48% of patients from Z1031 had high SET2,3 index prior to NeoET and significantly improved EFS. Distant and locoregional event rates were numerically lower, but rates of second primaries (including non-breast primaries) were not different.

Both components of SET2,3 contributed independently significant prognostic information in the Z1031 population, similar to our observations in the setting of NAC followed by adjuvant endocrine therapy (7). Furthermore, we observed from Z1031 that $SET_{ER/PR}$ was more prognostic in higher-risk tumors (low BPI) and, conversely, BPI was more prognostic in tumors with lower endocrine-related activity (low $SET_{ER/PR}$). This illustrates that measurement of baseline endocrine-related activity was complementary to assessment of baseline prognosis in the setting of adjuvant endocrine therapy.

Our observation that patients with high SET2,3 cancer were more likely to experience early pharmacodynamic response compares favorably with the rate of post induction Ki67 ≤ 10% results reported from the WSG ADAPT $HR^+/HER2^-$ trial of 84% in the 89 patients with recurrence score 0–11 and 76% in 225 of patients with recurrence score 12–25 (13, 14). Indeed, WSG ADAPT, ALTERNATE, the endocrine treatment arms of PALLET, and NeoMONARCH, provide additional opportunities to evaluate the SET2,3 assay for prediction of early pharmacodynamic response (Ki67 suppression; refs. 15–17). However, Ki67 suppression is probably not an optimal response surrogate for neoET combined with a cdk4/6 inhibitor because prevention of cell-cycle entry is a direct pharmacologic consequence. Indeed, the profoundly greater suppression of Ki67 observed from neoadjuvant trials when cdk4/6 inhibition was combined with endocrine treatment does not appear to predict survival benefit (16–19). Other response parameters may be needed for cdk4/6 inhibitors, such as change in intrinsic subtype score or residual cancer burden which are being considered in the CORALLEEN and NEOPAL trials (20, 21). Meanwhile, it is conceivable that SET2,3 (and/or $SET_{ER/PR}$) might identify patients who benefit from a cdk4/6 inhibitor combined with endocrine therapy (it has not yet been tested), and our plan would be to compare outcomes from a randomized trial using a survival endpoint.

Suppression of proliferation occurs quickly in response to NeoET and generally remains consistent during the course of NeoET treatments. For example, in the PALLET trial, Ki67 levels were lower at

Table 3. Multivariate Cox model for EFS that compared $SET_{ER/PR}$ and BPI (the component indices within SET2,3) measured in the tumor prior to NeoET.

Parameter pre-NeoET	Variable	Estimated HR	95% confidence interval	*P*
Baseline prognostic index	Dichotomous (at or above vs. below median)	0.47	0.31–0.72	0.0005
$SET_{ER/PR}$ index	Continuous (per unit of increase)	0.62	0.45–0.87	0.0051

Abbreviations: BPI, baseline prognostic index; pre-NeoET, prior to neoadjuvant endocrine therapy.

2 weeks but did not decrease further after 14 weeks in the letrozole and in the combined letrozole and palbociclib treatment arms (16). In general, tumors with low proliferation at baseline remain low after 2 to 4 weeks of endocrine therapy, whereas tumors with high proliferation at baseline may become low. The POETIC trial evaluated paired biopsies of operable $HR^+/HER2^-$ cancer before and after 2 weeks of letrozole in 2,235 patients (22). Using <10% to define low Ki67, 31% of cancers remained low Ki67, 18% remained high, 49% became low, and only 1% became high Ki67 (22). Similarly, a multigene proliferation score evaluated in Z1031 had lower scores in the baseline (pre-NeoET) biopsies from tumors that suppressed Ki67 to ≤10% after 2 to 4 weeks of endocrine therapy (5). We observed this effect with the baseline prognostic index, probably because BPI includes the proliferation gene aurora kinase A (*AURKA*; ref. 23). The other component index ($SET_{ER/PR}$) was designed to exclude genes obviously associated with proliferation yet had a significant but weaker association with Ki67 at baseline and at 2 to 4 weeks. This might be from indirect association between more differentiated cancer and lower proliferation. Importantly, high SET2,3 remained predictive of pharmacodynamic response in the tumor at 2 to 4 weeks after adjustment for the baseline Ki67 level in the tumor before treatment began, with adjusted ORs of 5.18 for Ki67 ≤ 10% and 2.78 for complete cell-cycle arrest.

Ki67 level after 2 to 4 weeks of endocrine therapy was prognostic in Z1031 and in the much larger POETIC preoperative window trial (5, 22). However, there was prognostic advantage for patients whose cancer had low Ki67 at diagnosis prior to treatment (22). An exploratory analysis of POETIC determined that prognostically optimal cut-off points for Ki67 would be at 20% at baseline and at 8% after 2 weeks of endocrine therapy (22). We did not study the 8% cut-off point in cohort B because we had seen similar results using cut-off points of 10% and 2.7%.

The proportion of women with *yp*Stage 0–I or PEPI = 0 pathologic response was not found to differ with respect to SET2,3 (high vs. low) or $SET_{ER/PR}$ (high vs. low) prior to NeoET. However, the proportion of women with *yp*Stage 0–I or PEPI = 0 was greater in those with high BPI prior to NeoET than those with low BPI. When dichotomized at the median, more favorable BPI was associated with approximately 3-fold increase in the percent of patients with either *yp*Stage 0–I (30% vs. 9%) or PEPI = 0 (33% vs. 11%). Pathologic response from NeoET is usually limited, because complete response (pCR) is rare and only a minority (20% to 25%) achieve *yp*Stage 0–I residual disease or cell cycle–arrested *yp*Stage I–II residual disease (PEPI = 0; refs. 1–3). PEPI is the better response measure for estimation of prognosis, with PEPI score 0 (group 1, PEPI-0) and PEPI score 1–3 (group 2) having better response than PEPI score ≥4 (group 3; refs. 5, 12, 24). One interpretation of this association with BPI is that lower pretreatment clinical stage increases the probability of having lower pathologic stage after NeoET, especially if treated with a shorter duration of NeoET. This is supported by a recent analysis of the literature and the National Cancer Database (NCDB) that reported patients with clinical node-positive disease had low probability of converting to node-negative status after NeoET (25). The rate of nodal conversion was 10% overall in the literature review and reached 15% in the NCDB analysis of patients with clinical N1 disease (25). Another interpretation is that lower proliferation at baseline (*AURKA* component of RNA4) increases the probability of achieving cell-cycle arrest at the end of NeoET that is necessary to be classified as PEPI = 0. Molecular subtypes are also associated with PEPI response. In Z1031, luminal A cancers had higher PEPI = 0 rate than luminal B (27% vs. 11%), although the PAM50 luminal subtypes were not prognostic (4). However, we propose that luminal subtypes combined with clinical stage would be prognostic, similar to what we observed with BPI (RNA4 with clinical stage; ref. 7). On the other hand, higher $SET_{ER/PR}$ index was associated with early pharmacodynamic response to NeoET and contributed independently to long-term EFS outcomes, but was not associated with PEPI = 0 rate, suggesting that it might provide additional prognostic information to molecular subtype and pathologic response, similar to the setting of NAC (7).

There are several limitations to this study. Although we used a predefined cut-off point for SET2,3 index, that cut-off point had been developed from patients who received NAC followed by adjuvant endocrine therapy and would have included some with more aggressive disease characteristics (7). Therefore, we conducted the threshold analyses to compare SET2,3 levels with response and patients' outcomes. Throughout the IQR of SET2,3 index, a higher SET2,3 value was generally associated with greater odds of pharmacodynamic response to endocrine therapy and improved EFS. Inspection of the threshold plots suggests that there may be an improved cut-off point at approximately 1.9, above which there are less significant odds of CCCA at 2 to 4 weeks or improved EFS over 8 years of follow-up. It is intuitively appealing to think that a cut-off point of SET2,3 index used to select patients for NeoET would be higher than the cut-off point of 1.77 that we had derived from the chemoendocrine treatment setting. However, the majority of patients from the Z1031 trial would have also received post-neoadjuvant adjuvant chemotherapy. Therefore, any inferred revision to the cut-off point based on these EFS results would still need to be interpreted pragmatically, such that patients with significant residual disease after NeoET might then be offered adjuvant chemotherapy.

Another limitation is that SET2,3 results were inferred from Agilent microarray gene expression measurements rather than the platform used to develop or to perform this test (7, 26). It is likely that this introduces some technical inaccuracy, although we had calibrated SET2,3 between genomic platforms to measure SET2,3 from a similar microarray platform to evaluate the I-SPY2 trial (7). We also note that cohort B followed and was informed by cohort A. Also, cohort B is a subset with 141 patients who had assessment of early pharmacodynamic response and PEPI = 0 pathologic response, and had reduced statistical power for the analyses of early pharmacodynamic response and PEPI pathologic response. In addition, NeoET in Z1031 was for 16–18 weeks duration and it is possible that longer duration of NeoET might further reduce disease burden. For example, it was reported from a cohort study that clinical objective response and pCR rates increased with longer duration of NeoET up to 1 year (27). Finally, quantitation of IHC stains for Ki67 might not be the most reproducible measure of proliferation, although best available practices were followed for the IHC analysis of Ki67 in these biopsy samples (5).

In conclusion, measurement of SET2,3 index prior to treatment predicted early pharmacodynamic response to NeoET and improved EFS, but not PEPI = 0. Both components of the SET2,3 index contributed independently significant prognostic information, demonstrating that measurements of endocrine activity, molecular subtype, and clinical stage of breast cancer are complementary for prognosis after adjuvant endocrine therapy. These results also indicate the potential for SET2,3 as an integral biomarker to enrich for patients with endocrine-sensitive breast cancer to consider neoadjuvant endocrine-based therapy, and it should undergo further evaluation in that clinical context.

Authors' Disclosures

V.J. Suman reports grants from NIH during the conduct of the study. T. Hoskin reports grants from Statistics and Data Center for the Alliance for Clinical Trials in Oncology NIH U10CA 180882 during the conduct of the study. C. Ma reports

personal fees from Gilead, AstraZeneca, Sanofi-Genzyme, Jacobio, Natera, Novartis, Inivata, Athenex, Bayer, Eisai, and Seattle Genetics; and grants from Puma and Pfizer outside the submitted work. K.K. Hunt reports other support from American College of Surgeons Oncology Group outside the submitted work; and is medical advisory board member of Armada Health and AstraZeneca and reports research funding to institution from Cairn Surgical, Eli Lilly & Co., and Lumicell. M.J. Ellis reports other support from Bioclassifier during the conduct of the study and other support from AstraZeneca outside the submitted work; in addition, M.J. Ellis has a patent for PAM50 issued, licensed, and with royalties paid from Veracyte. W.F. Symmans reports grants from Breast Cancer Research Foundation (BCRF-158) and Cancer Prevention and Research Institute of Texas (RP180712) during the conduct of the study and other support from Delphi Diagnostics, Inc, IONIS Pharmaceuticals, Eiger Biopharmaceuticals, personal fees from Merck and AstraZeneca, and grants from Pfizer outside the submitted work; in addition, W.F. Symmans has a patent for "Targeted Measure of Transcriptional Activity Related to Hormone Receptors," United States, Provisional Patent Application Serial No. 62/329,774 pending to Delphi Diagnostics Inc. No disclosures were reported by the other authors.

Authors' Contributions

V.J. Suman: Conceptualization, data curation, formal analysis, supervision, visualization, writing–original draft, writing–review and editing. **L. Du:** Data curation, formal analysis, investigation, methodology, writing–review and editing. **T. Hoskin:** Formal analysis, visualization, writing–original draft, writing–review and editing. **M. Anurag:** Data curation, methodology, writing–review and editing. **C. Ma:** Supervision, writing–review and editing. **I. Bedrosian:** Resources, writing–review and editing. **K.K. Hunt:** Resources, writing–review and editing. **M.J. Ellis:** Resources, supervision, writing–review and editing. **W.F. Symmans:** Conceptualization, supervision, funding acquisition, visualization, methodology, writing–original draft, project administration, writing–review and editing.

Acknowledgments

See https://acknowledgments.alliancefound.org. This work was presented in part at the 2021 American Society of Clinical Oncology (ASCO) Annual Meeting.

Research reported in this publication was supported by the NIH under award numbers U10CA180821, U10CA180882, and U24CA196171 (to the Alliance for Clinical Trials in Oncology); UG1CA233329, UG1CA233339; Cancer Prevention and Research Institute of Texas (CPRIT, RP180712, to W.F. Symmans, K.K. Hunt); Breast Cancer Research Foundation (BCRF-158, to W.F. Symmans). Also, NIH/NCI R01 CA095614 (M.J. Ellis) and the Baylor SPORE in Breast Cancer 2P50CA186784 (M.J. Ellis and V.J. Suman). M.J. Ellis is a Susan G. Komen Foundation Scholar emeritus, a McNair Scholar supported by the McNair Medical Institute at The Robert and Janice McNair Foundation, and a recipient of a CPRIT (Cancer Prevention and Research Institute of Texas) Established Investigator Award (RR140027). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Received January 8, 2022; revised April 18, 2022; accepted May 26, 2022; published first June 2, 2022.

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