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Tumor Intrinsic Subtypes and Gene Expression Signatures in Early-Stage *ERBB2/HER2*-Positive Breast Cancer

A Pooled Analysis of CALGB 40601, NeoALTTO, and NSABP B-41 Trials

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IMPORTANCE Biologic features may affect pathologic complete response (pCR) and event-free survival (EFS) after neoadjuvant chemotherapy plus *ERBB2/HER2* blockade in *ERBB2/HER2*-positive early breast cancer (EBC).

OBJECTIVE To define the quantitative association between pCR and EFS by intrinsic subtype and by other gene expression signatures in a pooled analysis of 3 phase 3 trials: CALGB 40601, NeoALTTO, and NSABP B-41.

DESIGN, SETTING, AND PARTICIPANTS In this retrospective pooled analysis, 1289 patients with EBC received chemotherapy plus either trastuzumab, lapatinib, or the combination, with a combined median follow-up of 5.5 years. Gene expression profiling by RNA sequencing was obtained from 758 samples, and intrinsic subtypes and 618 gene expression signatures were calculated. Data analyses were performed from June 1, 2020, to January 1, 2023.

MAIN OUTCOMES AND MEASURES The association of clinical variables and gene expression biomarkers with pCR and EFS were studied by logistic regression and Cox analyses.

RESULTS In the pooled analysis, of 758 women, median age was 49 years, 12% were Asian, 6% Black, and 75% were White. Overall, pCR results were associated with EFS in the ERBB2-enriched (hazard ratio [HR], 0.45; 95% CI, 0.29-0.70; $P < .001$) and basal-like (HR, 0.19; 95% CI, 0.04-0.86; $P = .03$) subtypes but not in luminal A or B tumors. Dual trastuzumab plus lapatinib blockade over trastuzumab alone had a trend toward EFS benefit in the intention-to-treat population; however, in the ERBB2-enriched subtype there was a significant and independent EFS benefit of trastuzumab plus lapatinib vs trastuzumab alone (HR, 0.47; 95% CI, 0.27-0.83; $P = .009$). Overall, 275 of 618 gene expression signatures (44.5%) were significantly associated with pCR and 9 of 618 (1.5%) with EFS. The *ERBB2/HER2* amplicon and multiple immune signatures were significantly associated with pCR. Luminal-related signatures were associated with lower pCR rates but better EFS, especially among patients with residual disease and independent of hormone receptor status. There was significant adjusted HR for pCR ranging from 0.45 to 0.81 (higher pCR) and 1.21-1.94 (lower pCR rate); significant adjusted HR for EFS ranged from 0.71 to 0.94.

CONCLUSIONS AND RELEVANCE In patients with *ERBB2/HER2*-positive EBC, the association between pCR and EFS differed by tumor intrinsic subtype, and the benefit of dual *ERBB2/HER2* blockade was limited to ERBB2-enriched tumors. Immune-activated signatures were concordantly associated with higher pCR rates and better EFS, whereas luminal signatures were associated with lower pCR rates.

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Supplemental content

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The highly aggressive *ERBB2/HER2*-positive breast cancer accounts for 20% of all breast tumors. However, the success of multiple *ERBB2/HER2*-targeting drugs has markedly improved outcomes. In *ERBB2/HER2*-positive early breast cancer (EBC), neoadjuvant treatment is now the standard of care given the surgical benefits of tumor downstaging[1] and the benefits of tailoring adjuvant anti-*ERBB2/HER2* drugs based on the presence of residual disease at surgery.[2] Pathologic complete response (pCR) at surgery has been associated with improved survival,[3] but a predictable quantitative relationship between pCR benefit and survival outcomes has been elusive.

In randomized neoadjuvant trials, dual *ERBB2/HER2*-targeting has been associated with higher pCR rates than single *ERBB2/HER2* targeting.[4-7] However, the magnitude of this effect has differed across trial populations and drugs, and neoadjuvant trials are typically underpowered for long-term outcomes. This was true of 3 phase 3 trials investigating dual (trastuzumab and lapatinib) vs single (trastuzumab or lapatinib) anti-*ERBB2/HER2* drugs added to chemotherapy: NeoALTTO (NCT00553358), CALGB 40601 (NCT00770809), and NSABP B-41 (NCT00486668). All 3 studies demonstrated higher pCR rates and better survival with dual therapy, which was statistically significant in NeoALTTO for pCR,[4] and CALGB 40601 for relapse-free survival.[8] In a trial-level meta-analysis including these 3 phase 3 studies and a similar phase 2 trial, Cher-LOB, dual blockade with trastuzumab plus lapatinib in combination with neoadjuvant chemotherapy significantly prolonged relapse-free and overall survival.[9] This result contrasts with the findings of the phase 3 adjuvant trial ALTTO,[10] in which patients with *ERBB2/HER2*-positive EBC treated with trastuzumab plus lapatinib blockade as adjuvant therapy had a 16% reduction in disease-free survival (DFS) with a hazard ratio (HR) that did not meet statistical significance. Dual therapy with trastuzumab plus pertuzumab in the phase 3 APHINITY trial resulted in a significant 24% improvement in invasive DFS HR and is the current standard of care in patients with high-risk disease.[11]

Differences in the effect of dual *ERBB2/HER2* blockade and robustness of the association of pCR with EFS could be partly explained by differences in tumor and microenvironment biology. *ERBB2/HER2*-positive EBC is not a singular biological entity; instead, it is characterized by heterogeneity of both cancer and immune cell components. At a tumor level, all the intrinsic molecular subtypes (ie, luminal A, luminal B, *ERBB2/HER2*-enriched, and basal-like) can be found within *ERBB2/HER2*-positive breast cancer tumors.[12] This intrinsic tumor heterogeneity has clinical implications. *ERBB2/HER2*-positive/*ERBB2*-enriched tumors have been systematically associated with higher pCR rates for *ERBB2/HER2*-targeted therapies,[13] and *ERBB2/HER2*-positive/ERBB2-enriched and *ERBB2/HER2*-positive/basal-like tumors have been associated with worse prognoses compared with *ERBB2/HER2*-positive/luminal tumors, particularly in those with residual disease.[8,14]

Similarly, the immune microenvironment is both predictive and prognostic. Tumor-infiltrating lymphocytes (TILs), which correlate strongly with T-cell expression signatures, have been significantly associated with higher pCR rates and EFS in multiple *ERBB2/HER2*-positive neoadjuvant studies.[7,8,15-25] In a recent analysis, immune gene signatures appear more valuable than TILs in pCR prediction.[26] Another important finding is that immune signatures and TILs are associated with both higher pCR rates and longer EFS, unlike intrinsic subtypes, which often work in opposite directions for predicting response and survival.[8]

We hypothesized that the biological tumor and immune heterogeneity of *ERBB2/HER2*-positive EBC contribute to the inconsistent results coming from different neoadjuvant and adjuvant clinical trials. In this analysis, we examined how intrinsic subtype, immune activation status, and other gene expression signatures contribute to pCR and EFS, and the benefit of dual therapy with trastuzumab and lapatinib compared with single-agent trastuzumab, by performing individual patient-level biomarker analysis of 3 phase 3 clinical trials with similar designs: NeoALTTO,[4,15,21,27-30] CALGB 40601,[7,8,26] and NSABP B-41.[6,14,31,32]

Key Points

Question What is the quantitative association between pathologic complete response (pCR) and event-free survival (EFS) by intrinsic subtype and other gene expression signatures in patients with *ERBB2/HER2*-positive early breast cancer (EBC) treated in the neoadjuvant setting?

Findings In this retrospective pooled analysis of 3 randomized clinical trials including 1289 patients with *ERBB2/HER2*-positive EBC, the association between pCR and EFS differed by tumor intrinsic subtype, and the benefit of dual *ERBB2/HER2*-blockade was limited to ERBB2-enriched tumors. Immune-activated signatures were associated with higher pCR rates and better EFS, whereas luminal signatures were associated with lower pCR rates.

Meaning Intrinsic subtype and immune gene expression biomarkers may help guide personalized treatment in patients with *ERBB2/HER2*-postive EBC.

Methods

Neoadjuvant Trials: Study Designs and Patients

All 3 phase 3 trials have had pCR, correlative, and/or survival end points published previously (NeoALTTO,[4,15,21,27-30] CALGB 40601,[7,8,26] NSABP B-41[6,14,31,32]). All trials involved previously untreated patients with early *ERBB2/HER2*-positive breast cancer randomized to chemotherapy with single trastuzumab, single lapatinib, or dual trastuzumab and lapatinib anti-*ERBB2/HER2* therapy (eFigure 1A in Supplement 1). Trial differences included a 6-week lead-in phase of the randomized anti-*ERBB2/HER2* agent(s) in the NeoALTTO trial,[4,15,21,27-30] all chemotherapy given as neoadjuvant therapy in B-41, neoadjuvant durations varying from 16 weeks (CALGP 40601) to 28 weeks (B-41), and that the adjuvant anti-*ERBB2/HER2* therapy was as randomized in the NeoALTTO trial but was single-agent trastuzumab in the other 2 trials.

To homogenize the clinical outcomes from the 3 clinical trials, for this patient-level pooled analysis, pCR was defined

as the absence of invasive tumor cells in the breast (ypT0/is). We used EFS for long-term outcome, defined as the time from randomization to the event (ie, local recurrence, regional recurrence, distant recurrence, nonbreast second primary tumors, contralateral invasive breast cancer, and death of any cause). A slight difference in the event definitions between the 3 clinical trials included progressions during the neoadjuvant phase, which were regarded as events in the CALGB 40601 and NeoALTTO trials but not in the B-41 trial. However, only 3 patients from the NeoALTTO and 5 from the B-41 trials progressed during the neoadjuvant phase. In the intention-to-treat (ITT) cohort, 10 patients from NSABP B-41 did not have EFS events and/or time information collected.

Ethics committee and relevant health authorities at each participating site approved the NeoALTTO, CALGB 40601, and NSABP B-41 studies, and all patients provided written informed consent, including future biomarker research.

Tumor Gene Expression Analyses

Gene expression profiles from pretreatment core biopsies were obtained from 249 of 455 participants (54.7%) in the NeoALTTO, 264 of 305 participants (86.6%) in the CALGB 40601, and 245 of 529 participants (46.3%) in the NSABP B-41 trials, respectively (CONSORT diagram, eFigure 1B in Supplement 1). The tumor preservation methods, RNA extraction, RNAseq library preparation, sequencing parameters, bioinformatic algorithms, and data preprocessing are summarized in the eMethods in Supplement 1. A principal component analysis (PCA) plot before and after the batch effect correction can be found in eFigure 2 in Supplement 1.

For the 3 studies, intrinsic subtypes and a collection of 618 gene expression signatures (GES) representing diverse cell types and biologic pathways were obtained from RNAseq gene expression data as described previously[8,26] (eMethods, eTable 1 in Supplement 1).

Statistical Analysis

Comparisons of differences in baseline clinicopathologic variables among the trials were made using a Wilcoxon rank-sum for continuous variables and χ^2 or Fisher exact tests for categorical variables. Proportions and *P* values are provided. For the survival analyses, the 5-year EFS proportions for each group were estimated using the Kaplan-Meier method. The association between clinical and genomic biomarkers with pCR and EFS was assessed using univariable and multivariable logistic and Cox regression models, respectively. Clinical variables considered for multivariable models included clinical trial (ie, CALGB 40601, NeoALTTO, and NSABP B-41, where CALGB 40601 was the reference group), treatment arm (ie, trastuzumab, trastuzumab plus lapatinib, or lapatinib, where trastuzumab was the reference arm), hormone-receptor status (hormone receptor positive vs hormone receptor negative), clinical tumor size (T1-T2 vs T3-T4a-c), and clinical node involvement (node positive vs node negative). Inflammatory breast cancer was excluded in all trials. All Cox models were stratified by clinical trial. Odds ratios (ORs), hazard ratios (HRs), and 95% CIs were calculated for each variable. The significance level was set to a 2-sided α of .05, and *P* values were adjusted for multiple testing using the Benjamini and Hochberg method to control the false discovery rate. To avoid a potential guarantee time bias in the multivariable EFS models including pCR status, we performed a 30-week landmark analysis. The landmark subpopulation included only patients without events and being followed up at 30 weeks after randomization.[27,33]

All the analyses were based on the study clinical database frozen on May 26, 2016, in the NeoALTTO trial, on June 10, 2021, in the CALGB 40601 trial, and on December 31, 2016, in the NSABP B-41 trial, and were performed using R (version 3.5.2; R Foundation for Statistical Computing) and Python statistical software (version 3.6; Python Software Foundation). Data analyses were performed from June 1, 2020, to January 1, 2023.

Results

Clinicopathologic Characteristics and Efficacy Analysis in the ITT Cohort

There were 1289 patients with *ERBB2/HER2*-positive EBC included. Although generally similar, several baseline clinicopathologic features differed among the 3 trials, including larger tumors, more node-positive, and a higher proportion of Asian participants in the NeoALTTO trial (eTable 2 in Supplement 1).

In the ITT population, a multivariable analysis for pCR prediction found that patients treated with trastuzumab plus lapatinib had significantly higher pCR rates than those treated with trastuzumab (adjusted OR [aOR], 1.80; 95% CI, 1.36-2.39; *P* < .001), with no significant differences between the lapatinib and trastuzumab arms (eTable 3 in Supplement 1). The Kaplan-Meier estimates of 5-year EFS by treatment were 83%, 79%, and 73% for the lapatinib and trastuzumab, trastuzumab, and lapatinib arms, respectively (eFigure 3 in Supplement 1), a difference that was not significant in a multivariable Cox analysis (lapatinib and trastuzumab vs trastuzumab: adjusted HR [aHR], 0.74; 95% CI, 0.54-1.01; *P* = .056) (eTable 4 in Supplement 1).

When comparing the EFS among the 3 studies, the NeoALTTO trial had significantly worse EFS outcomes than the CALGB 40601 trial (HR, 1.92; 95% CI, 1.39-2.66; *P* < .001) (eFigure 4 in Supplement 1). In the overall cohort of patients that were treated with trastuzumab in the neoadjuvant setting (n = 887), patients with pCR had a significantly better EFS outcome than patients with residual disease in a multivariable model stratified by clinical trial and adjusted by treatment arm, hormone receptor status, tumor size, and node status (aHR for pCR vs residual disease: 0.47; 95% CI, 0.34-0.66; *P* < .001). The Kaplan-Meier estimates of 5-year EFS were 88% for pCR and 74% for patients with residual disease (eFigure 5A in Supplement 1). Similar results were observed when the landmark analysis was performed (n = 856) (eFigure 5B in Supplement 1).

Similar distributions of local, distant, and organ sites of recurrence or death comprising the EFS events were seen across the 3 trials (eFigure 6 in Supplement 1). Patients treated only with lapatinib generally experienced more distant recur-

Table. Comparison of Baseline Clinicopathologic Characteristics of the Patients From the NSABP B-41, CALGB 40601, and NeoALTTO Trials in the 758 Patients in the RNAseq Cohort

Variable	No. (%) NSABP B-41 (n = 245)	C40601 (n = 264)	NeoALTTO (n = 249)	*P* value[a]
Age, median (IQR), y	48 (42-56)	49 (41-56)	50 (40-55)	.85
Menopause status				
Postmenopausal	110 (44.9)	102 (38.6)	117 (47.0)	.14
Premenopausal	135 (55.1)	162 (61.4)	132 (53.0)	
Race				
Asian	10 (4.1)	16 (6.1)	68 (27.3)	<.001
Black	24 (9.8)	21 (8.0)	4 (1.6)	
Other	7 (2.9)	14 (5.3)	22 (8.8)	
White	204 (83.3)	213 (80.7)	155 (62.2)	
HR status				
HR negative	104 (42.4)	110 (41.7)	115 (46.2)	.55
HR positive	141 (57.6)	154 (58.3)	134 (53.8)	
Clinical tumor size				
T1-T2	168 (68.6)	181 (68.6)	148 (59.4)	<.001
T3-T4	77 (31.4)	61 (23.1)	101 (40.6)	
Unknown	0	22 (8.3)	0	
Tumor size by physical examination, median (IQR), cm	4 (3-6)	4 (3-5)	4 (3-8)	.07
Clinical status of lymph nodes				
N positive	129 (52.7)	136 (51.5)	181 (72.7)	<.001
N negative	116 (47.3)	113 (42.8)	67 (26.9)	
Unknown	0	15 (5.7)	1 (0.4)	
Treatment arm				
Trastuzumab	86 (35.1)	104 (39.4)	77 (30.9)	.004
Trastuzumab and lapatinib	75 (30.6)	103 (39.0)	84 (33.7)	
Lapatinib	84 (34.3)	57 (21.6)	88 (35.3)	

Abbreviations: HR, hormone receptor; T1-T2, tumor size ≤50 mm; T3-T4, tumor size >50 mm; N, lymph nodes infiltration.

[a] Kruskal-Wallis rank sum test; Pearson χ^2 test.

rences than patients treated with trastuzumab or trastuzumab plus lapatinib, except for brain metastasis, which was more frequent in the trastuzumab arm, suggesting activity of lapatinib in preventing brain relapses as has been noted with other anti-*ERBB2/HER2* small molecules (eFigure 7 in Supplement 1).[34]

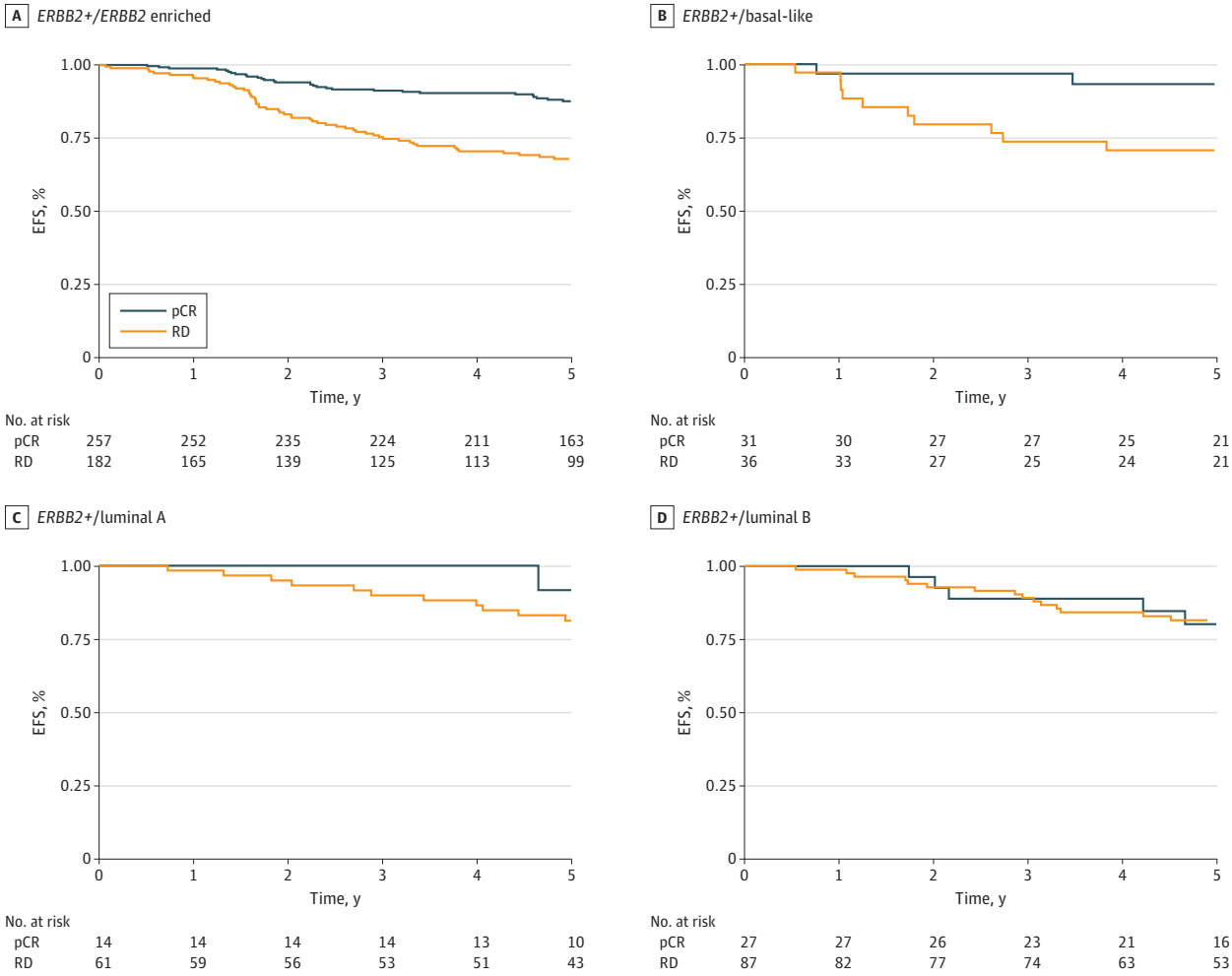
Clinical Implications of the Intrinsic Subtypes in the RNAseq Cohort

The CALGB 40601 trial had a higher proportion of patients represented in the RNAseq cohort, whereas the NSABP B-41 trial was more represented in the ITT cohort. Otherwise, there were no significant differences among the clinicopathologic characteristics, response, and EFS survival outcomes of the parent ITT and the RNAseq cohorts (eTable 5 in Supplement 1). In the RNAseq cohort, several baseline clinicopathologic features differed among the 3 trials, including larger tumors, more node positive, and a higher proportion of Asian participants in the NeoALTTO trial (**Table**).

When analyzed for RNAseq-based tumor intrinsic subtype on the combined cohort, most tumors were *ERBB2* enriched (57.9%), followed by luminal B (15.0%), luminal A (9.9%), basal-like (8.8%), and normal-like (8.3%). There were no significant differences in the intrinsic subtype distribution by study. Subtype distribution significantly differed by hormone-receptor status (eTable 6 in Supplement 1). There were no significant differences in the hormone-receptor status distribution by locoregional and distant relapse location (eFigure 8A in Supplement 1). However, there were significant differences in the intrinsic subtype distribution by the site of relapse: only patients with *ERBB2/HER2*-positive/ERBB2-enriched and *ERBB2/HER2*-positive/basal-like tumors had brain metastases. Importantly, 5 of the 55 brain metastases were from hormone-receptor–positive tumors; all had a nonluminal subtype (1 basal-like and 4 *ERBB2* enriched). *ERBB2/HER2*-positive/luminal tumors developed more bone and visceral metastasis (eFigure 8B in Supplement 1).

The association between pCR and EFS was different by tumor intrinsic subtype. In a multivariable Cox model stratified by study and adjusted by treatment arm, pCR status was significantly associated with EFS in patients with *ERBB2/HER2*-positive/*ERBB2*-enriched (aHR, 0.45; 95% CI, 0.29-0.70; *P* < .001) and *ERBB2/HER2*-positive/basal-like tumors (aHR, 0.19; 95% CI, 0.04-0.86; *P* = .03), but not in patients with *ERBB2/HER2*-positive/luminal A or B disease (**Figure 1**). Similar results were obtained when performing a landmark analysis (eTable 7 in Supplement 1). In a stratified univariable Cox model, a significant EFS benefit of dual trastuzumab and lapatinib vs single trastuzumab *ERBB2/HER2*-blockade was found only in patients with *ERBB2/HER2*-positive/*ERBB2*-enriched disease (aHR for trastuzumab and lapatinib vs trastuzumab alone, 0.48; 95% CI, 0.27-0.83; *P* = .009) but not in patients with basal-like or luminal *ERBB2/HER2*-positive EBC (**Figure 2**).

Figure 1. Kaplan-Meier Curves of the Association of Pathologic Complete Response (pCR) With Event-Free Survival by Tumor Intrinsic Subtype



Kaplan-Meier event-free survival (EFS) proportions at 5 years are provided. Cox regression models were stratified by clinical trial. Patients with normal-like tumors were removed from the analysis. RD indicates residual disease.

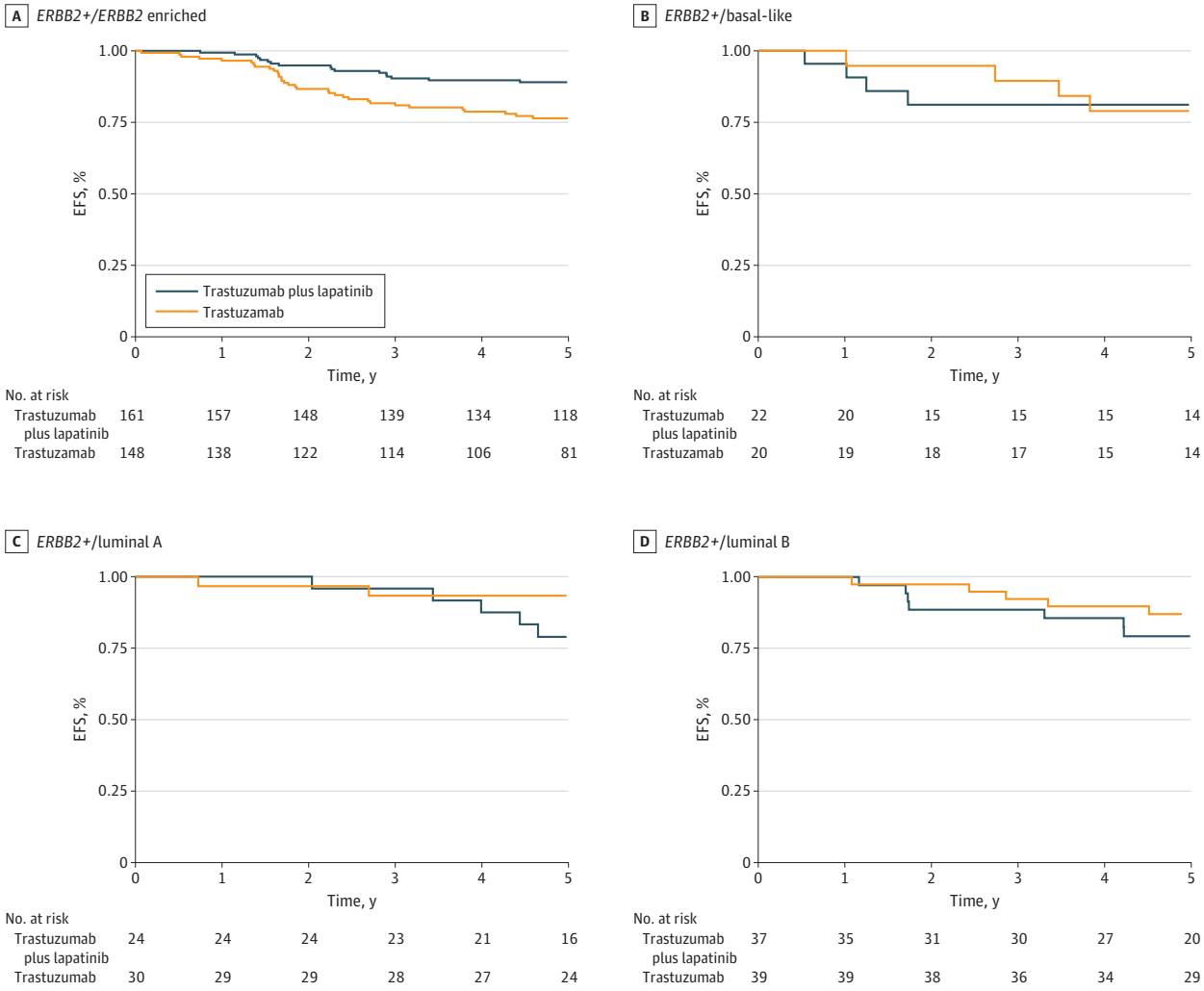
pCR and EFS Biomarkers Across Individual Trials and in the Combined Cohort

Using the combined cohort, 275 of 618 gene expression signatures (44.5%) were significantly associated with pCR, and 8 biomarkers were significantly associated with pCR individually in each trial (eTable 8 in Supplement 1). A selection of the signatures more consistently and strongly associated with pCR is shown in **Figure 3**A. In general, *ERBB2/HER2*, proliferation, and immune-related signatures were associated with higher pCR rates. Some differences were observed between the studies at an immune signature level; for example, B-cell-related signatures were more associated with pCR in the CALGB 40601 trial, whereas T-cell-related signatures were more associated with pCR in the NeoALTTO trial. As expected, luminal-related signatures were associated with lower pCR rates across the 3 studies and the combined cohort.

In multivariable Cox regression analysis, only 9 of 618 signatures (1.5%), all immune, were significantly associated with EFS in the combined cohort when *P* values were adjusted by multiplicity (eTable 9 in Supplement 1). Some of the signatures strongly associated with EFS are represented in Figure 3B. Concordant with their association with pCR, B-cell-related immune gene expression signatures were significantly associated with longer EFS, whereas vascular, proliferation, and metastasis signatures were associated with worse prognosis, although these associations were no longer significant when adjusted for multiple comparisons (eTable 9 in Supplement 1).

Significantly better prognosis was seen in the 409 patients with residual disease if these tumors were HR-positive at baseline (aHR, 0.50; 95% CI, 0.34-0.74; *P*<.001) or luminal expression subtypes (luminal vs HER2-enriched aHR, 0.55; 95% CI, 0.35-0.86; *P* = .01) (eFigure 9A and 9B in Supplement 1). Among patients with residual disease, those with higher immune infiltration at baseline showed a significantly better prognosis. In contrast, high MAPK activation pathway signature levels and the gene expression of *ERBB3* were significantly associated with worse outcomes, although these associations were not signifi-

Figure 2. Kaplan-Meier Curves of the Association of the Treatment Arm With Event-Free Survival by Tumor Intrinsic Subtype



Kaplan-Meier event-free survival (EFS) proportions at 5 years are provided. Cox regression models were stratified by clinical trial. Patients with normal-like tumors and treated with lapatinib only were removed from the analysis.

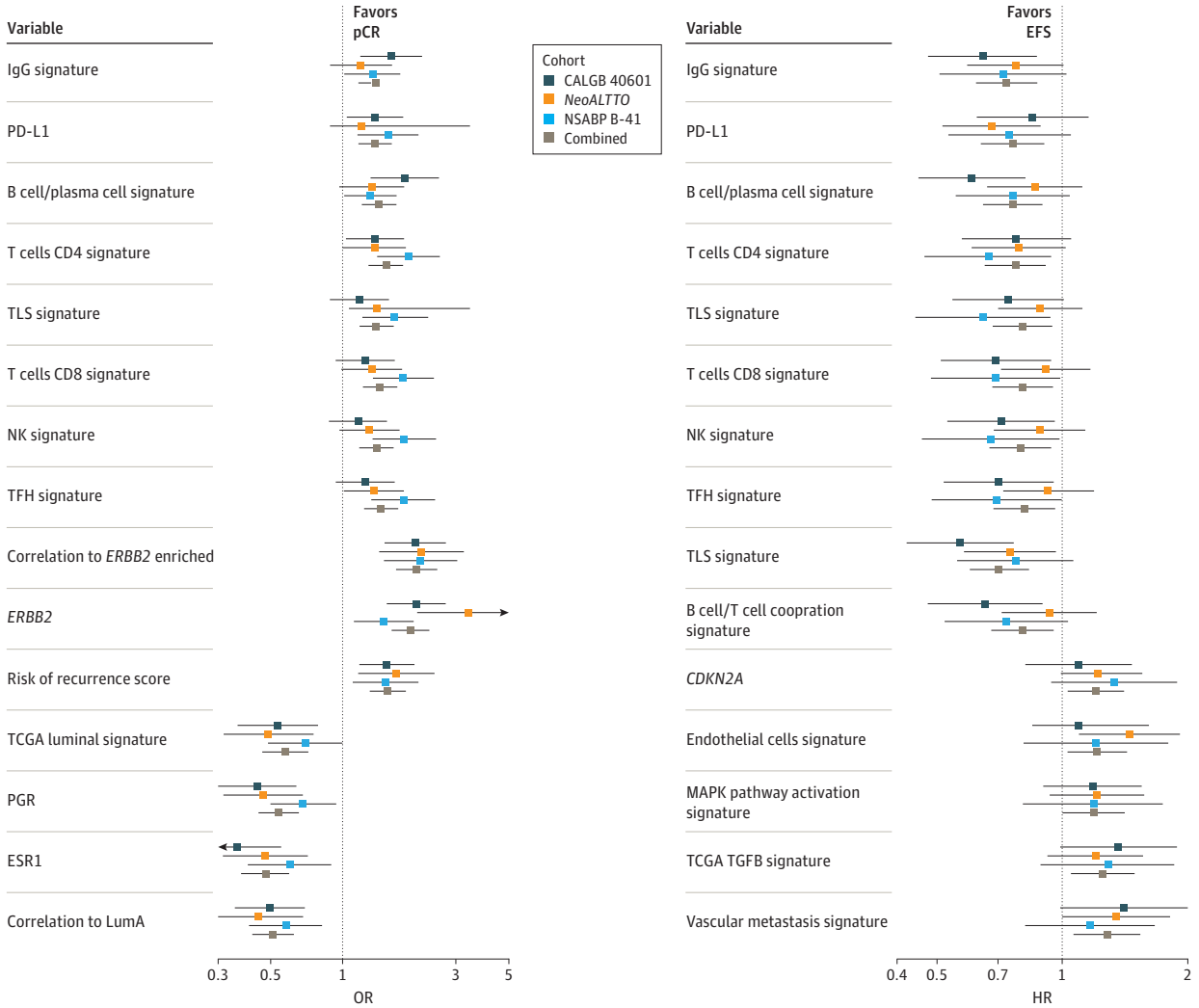
cant when adjusted for multiple comparisons (eFigure 9C and eTable 10 in Supplement 1).

Discussion

This pooled analysis of 3 phase 3 clinical trials with similar designs (ie, NSABP B-41, CALGB 40601, and NeoALTTO) illustrates how the association between pCR and survival in *ERBB2/HER2*-positive EBC varies by genomic intrinsic subtype and is only significant in patients with *ERBB2/HER2*-positive/*ERBB2*-enriched and *ERBB2/HER2*-positive/basal-like tumors. We also found a significant pCR and EFS benefit of dual anti-*ERBB2/HER2* therapy limited to patients with *ERBB2*-enriched tumors, which comprise approximately 60% of *ERBB2/HER2*-positive EBC.

Prognostic and predictive biomarkers are needed in *ERBB2/HER2*-positive EBC to guide the future tailoring of treatment strategies. However, one of the biggest obstacles in biomarker research is the lack of validation. In this individual-level pooled analysis of 758 patients, we were able to test hundreds of gene expression signatures for pCR prediction and outcome prognostication, even though these studies were not powered individually for survival outcomes. *ERBB2* amplicon genes, proliferation, and immune signature levels at baseline were significantly associated with pCR rates in each trial and the combined cohort. In contrast, luminal genes and signatures were significantly associated with lower pCR rates but did not appear to have EFS implications in this setting. Immune signatures were also significantly associated with better EFS outcomes in the combined cohort. These results support our previous findings,[8] suggesting that the combination of tumor (ie, *ERBB2* amplicon genes, proliferation, and luminal signatures) and immune-related biomarkers provide essential prognostic information to stratify patients with *ERBB2/HER2*-positive EBC in different groups that could benefit from different treatment strategies; some newer commer-

Figure 3. Forest Plots Showing the Association of Gene Expression Biomarker Levels at Baseline With Pathologic Complete Response (PCR) and Event-Free Survival (EFS)



A selection of the most interesting and consistent biomarkers across the 3 studies and the combined cohort is shown. All the models have been stratified by clinical trial and adjusted by tumor size, hormone receptor status, and clinical node involvement. The whiskers indicate the 95% CIs. HR indicates hazard ratio; IgG, immunoglobulin G; OR, odds ratio; PD-L1, programmed death-ligand 1.

cially available predictors already integrate these elements into a single assay.[24]

Limitations

This study has several limitations. First, lapatinib is only approved in the metastatic setting but not for *ERBB2/HER2*-positive EBC treatment. Second, even though all 3 trials aimed to test a common hypothesis (ie, if dual *ERBB2/HER2*-blockade with trastuzumab plus lapatinib was better thantrastuzumab alone in terms of pCR), the designs were slightly different: in the NSABP B-41 trial, all chemotherapy was administered before surgery, whereas in the NeoALTTO and CALGB 40601 trials only the taxane component was preoperative, whereas the anthracycline-based regimen was administered after surgery. However, several associations with pCR were consistent across the 3 cohorts. Third, in the NeoALTTO trial, there was a brief induction phase of 6 weeks with only *ERBB2/HER2*-targeted drugs, and T was administered for 12 weeks, compared with 16 weeks in NSABP B-41 and CALGB 40601. Moreover, in the NeoALTTO adjuvant phase, the *ERBB2/HER2*-blockade treatment was the same as in the induction phase and not the standard-of-care trastuzumab for 1 year through most of the trial. The variations in treatment as well as the higher proportion of high clinical risk patients may have contributed to the worse EFS seen in NeoALTTO compared with the other trials. All the models performed in our study have been adjusted and/or stratified by the clinical trial to mitigate these differences and adjusted for key clinical variables. Fourth, the proportions of T4a-c tumors within each study were not studied in this pooled analysis. However, the tumor size assessed by physical examination was not significantly different among the 3 studies. Fifth, even when pooling together patients from 3 trials, the number of EFS events is limited when dividing the study cohort

by subgroups, which may result in inadequate statistical power for certain statistical comparisons. Finally, there was a slight difference in the EFS event definition in NSABP B-41, in which progression during the neoadjuvant phase was not counted as an event; however, this was an uncommon occurrence, and we did not find variation in local and distant event proportions across the 3 studies.

Conclusions

This analysis shows for the first time 2 main clinical implications of tumor intrinsic subtype differences within *ERBB2/HER2*-positive EBC, demonstrating that the association of EFS with pCR after chemotherapy plus *ERBB2/HER2* targeting is seen only in patients with ERBB2-enriched and basal-like tumors and only ERBB2-enriched patients benefit from dual neoadjuvant *ERBB2/HER2*-blockade with trastuzumab and lapatinib. Common biomarkers of pCR and EFS included *ERBB2* amplicon genes and immune gene signatures, whereas in luminal tumors, pCR was less common but had little prognostic implication. Our results highlight the need to incorporate intrinsic subtype and immune gene expression biomarkers to guide personalized treatment in *ERBB2/HER2*-positive EBC.

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