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Association of Stromal Tumor-Infiltrating Lymphocytes With Recurrence-Free Survival in the N9831 Adjuvant Trial in Patients With Early-Stage HER2-Positive Breast Cancer

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Abstract

Importance—Tumor-infiltrating lymphocytes at diagnosis are reported to be prognostic in triple-negative breast cancer.

Objective—Here we evaluate the association of stromal tumor infiltrating lymphocytes (STILs) with recurrence-free survival (RFS) in N9831 HER2-positive patients treated with chemotherapy or chemotherapy plus trastuzumab.

Design—H&E tumor slides from patients on N9831 Arm A (standard AC→T chemotherapy) and Arm C (concurrent chemotherapy with trastuzumab) were assessed for STILs. STILs were quantitated in deciles and ≥60% STILs was used for the pre-specified categorical cutoff. The association between STILs and recurrence-free survival (RFS) was evaluated with Cox models.

Setting—Academic medical center

Participants—Tumor specimens from patients with early stage HER2+ breast cancer.

Intervention(s) for clinical trials or exposure(s) for observational studies—None.

Main outcome measures—Stromal tumor infiltrating lymphocytes (STILs) and their association with relapse-free survival.

Results—489 pts from Arm A and 456 pts from Arm C were assessed with a median follow-up of 4.4 years. The 10 year Kaplan-Meier estimates for RFS in Arm A were 90.9% and 64.5% for patients with high STILs and low STILs, respectively (HR 0.23; 95%CI: 0.073 to 0.73; p=0.013).

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The 10 year estimates for RFS in Arm C were 80.0% and 80.1% for patients with high STILs and low STILs, respectively (HR 1.26; 95%CI: 0.5 to 3.2; p=0.63). The test for interaction between trastuzumab treatment and STILS status was statistically significant (p=0.026). In a multivariable analysis, STILs status remained significantly associated with RFS in Arm A and not significantly associated in Arm C (interaction p=0.042).

Conclusions and relevance—The analysis of N9831 patients found that STILs were prognostically associated with RFS in patients treated with chemotherapy alone, but not prognostically associated with RFS in patients treated with chemotherapy plus trastuzumab. High STILS were predictive of lack of trastuzumab benefit in contrast to a previously reported association between increased STILs and increased trastuzumab benefit in HER2 positive patients.

Trial Registration—Trial registration information: Clinicaltrials.gov, NCT00005970, https://clinicaltrials.gov/show/NCT00005970

INTRODUCTION

The presence of dense lymphocytic infiltrates in breast carcinoma has long been recognized by breast histopathologists.[1] The term medullary carcinoma was first employed back in 1949 to describe a high grade breast carcinoma growing in anastomosing sheets comprised of large cells with numerous mitoses and an “intimate” stromal lymphoid infiltrate that was associated with a better than average prognosis.”[1] The association of dense stromal lymphocytic infiltrates characteristic of medullary carcinoma and a good prognosis continued to be documented though-out the twentieth century; however, the etiology of this better prognosis remained uncertain.[2–4] Medullary carcinomas are by definition estrogen receptor negative. Microarray-based comparative genomic hybridization studies examining the enriched tumor DNA of medullary carcinoma show that medullary breast carcinomas share common genomic alterations with basal-like carcinomas, the most frequent being 1q and 8q gains and X losses. However, medullary breast carcinomas appear to be a distinct entity within the basal-like spectrum characterized by a higher proportions of genome copy number aberrations than basal carcinomas and recurrent 10p, 9p and 16q gains, 4p losses, and 1q, 8p, 10p and 12p amplicons and most importantly are associated with “better prognosis”.[5,6]

Today, the role of the immune system in breast cancer development and outcome is undergoing significant study, especially in the setting of triple negative (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Recent retrospective analyses have demonstrated a prognostic association of stromal tumor infiltrating lymphocytes (STILs) with outcome in patients receiving adjuvant or neoadjuvant chemotherapy for triple negative breast cancer[7–13]. These studies have confirmed that TILs are most frequently found in highly proliferative TNBC and to a slightly lesser degree HER2–positive breast cancer. Their presence at diagnosis is associated with pathologic response to neoadjuvant therapy, disease-free (DFS) and overall survival (OS) after adjuvant chemotherapy[8–10,14,15]. Subset analysis of HER2-positive breast cancers from the BIG 02-98 adjuvant study has documented higher levels of TILs were significantly associated with improved survival in patients who did not receive taxane[10]. Furthermore, analysis of HER2+ cancers from patients enrolled in FinHER adjuvant study has suggested that STILs

are predictive of benefit to adjuvant trastuzumab[14]. However, these data from FinHER trial are based on only 209 patients randomized to chemotherapy +/− trastuzumab and associated with small number of events (N=49 events) between the two treatment groups. The goal of the current study was to determine whether the data from FinHER could be validated in a larger adjuvant trial with the standard 1 year of trastuzumab such as N9831.

Herein we describe a prospective-retrospective exploratory analysis of STILs and recurrence-free survival (RFS) outcome in patients enrolled in the N9831 adjuvant trial which evaluated chemotherapy alone or chemotherapy with trastuzumab in patients with early stage HER2-positive breast cancer. Guidelines followed for our analysis included those recommended by Simon et al[16] and the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies)[17]. Archived specimens collected at baseline for patients with centrally tested HER2-positive breast cancer enrolled in Arms A and C of N9831 (chemotherapy alone, Arm A vs. chemotherapy plus concurrent trastuzumab, Arm C) were evaluated. Arm C represents the current standard of care, and this arm exhibited maximum difference in RFS after trastuzumab, compared to RFS after chemotherapy alone (Arm A 10 year RFS =67.1%; Arm C 10 year RFS =79.7%)[18]. The purpose of our analyses was to determine whether STILs is predictive of RFS for HER2+ patients treated with trastuzumab.

PATIENTS AND METHODS

Study Patients

The N9831 Phase III randomized trial included 3505 women with histologically confirmed node-positive or high-risk node-negative HER2-positive invasive breast cancer. Eligible patients were randomly assigned to doxorubicin and cyclophosphamide (doxorubicin/cyclophosphamide or AC) followed by weekly paclitaxel (control arm, Arm A); AC followed by weekly paclitaxel followed by trastuzumab (sequential arm, Arm B); or AC followed by weekly paclitaxel plus trastuzumab followed by trastuzumab alone (concurrent arm, Arm C). Results of the different arms of N9831 were published in 2011, demonstrating that although each trastuzumab-containing arm led to statistically significant better disease-free survival compared to chemotherapy alone, the largest difference was observed in the Arm C versus Arm A comparisons. The present analyses included only patients randomly assigned to Arms A or C, enrolled from May 25, 2000 through April 25, 2005. Radiation and/or hormonal therapy were administered after the completion of chemotherapy, as indicated. Patient accrual occurred from 2000–2005; follow-up is ongoing although the primary and secondary clinical objectives have been published[18,19]. Baseline ER, PR, and HER2+ status was assessed according to protocol guidelines as described previously[18].

Pathologic Analysis of STILs

Histopathologic analysis of the percentage of STILs was prespecified and performed using a single H&E (hematoxylin and eosin) stained section from each tumor using the criteria by Loi et al, Denkert et al, and Adams et al[9–11,20]. STILs were defined as the percentage of tumor stroma containing infiltrating lymphocytes that were not in direct contact with tumor cells from an assessment of the entire tumor containing area of the section. Areas of non-invasive cancer or crush artifacts were not included in the analyses. The STIL data were

collected as deciles. Specifically, each specimen was determined to consist of 0–9%, 10–19%, 20–29%, 30–29%, 40–49%, 50–59%, 60–69%, 70–79%, 80–89%, or 90–100% STILs. A priori, tumors were classified as lymphocyte predominant breast cancer (LPBC) if they consisted of ≥ 60% STILs consistent with Denkert et al[15]. This histopathological review was conducted in tandem by two pathologists for the first 100 cases (F.L.B., H.B.) followed by a single pathologist (H.B.). 12 samples were randomly selected from each of the 10 STIL bins (N=120) and an independent pathologist (S.B.) reviewed them using the same published criteria but without any teaching set[15,20]. All were blinded to the patient's treatment assignment, tumor staging, and clinical outcome.

Statistical Analyses

Differences in continuous variables between groups were evaluated with a t-test or a Wilcoxon rank sum test if the distribution was skewed. Differences in categorical variables were evaluated with a chi-square test. The inter-rater agreement was assessed with a weighted Kappa statistic where 0 indicates no agreement and 1 indicates perfect agreement. Recurrence-free survival was defined as time from randomization until recurrent disease (local, regional, or distant recurrence of breast cancer). Patients who had not experienced a disease recurrence at the time of last follow-up or death were censored at the date of last follow-up or death. Kaplan-Meier curves were used to summarize the RFS experience and the curves were compared with a log-rank test. A Cox proportional model was used to determine whether there was an interaction between LPBC status and treatment arm in terms of an association with RFS. The model contained the main effects (treatment arm and LPBC status) as well as the interaction term of LPBC status*treatment arm. Since the interaction term was significant, separate analyses were done for each treatment arm. Univariable and multivariable Cox models were used to generate hazard ratios and corresponding 95% confidence intervals (CIs) for determining associations between variables of interest and RFS. A secondary analysis was performed using the decile levels as continuous measurements in place of LPBC status within the Cox models; the coding of the deciles was 0–9% coded as 1, 10%–19% coded as 2, 20–29% as 3, etcetera. This recoded variable was treated as continuous.

This was an unplanned ad hoc analysis with a pre-determine sample size that resulted from the number of patients enrolled in the trial who had consented to the use of their specimen for analysis and who sufficient tumor tissue for analysis. Given the sample size for this analysis was pre-determined by the number of patients who were consented and who had materials for correlative sciences, a power calculation was not done. Instead, we provide 95% CIs for all the results so that the reader can decided whether the intervals contain values that would be considered clinically significant in the cases where the results were not found to be significant.

RESULTS

Patient characteristics

There was a total of 2027 eligible patients enrolled on Arms A and C of N9831: 1081 on Arm A and 946 on Arm C. A subset of 945 patients was included in the STILs analysis.

These patients were eligible and had provided sufficient tissue for analysis (see Figure 1). A comparison between those patients included in the STILs analysis and those not revealed the two groups differed significantly with respect to race with a greater percentage of white patients included in the STILs analysis (81% in the cohort not included and 88% in the cohort included, p = 0.0001). The two groups did not differ significantly on other baseline variables (eTable1). In the parent study (N = 2027; total number of events = 352) the HR comparing the RFS of arm C to arm A was 0.51 (95% CI: 0.41 to 0.64, p < 0.0001; 240 events in 1081 patients for Arm A; 112 events in 946 patients for Arm C). The HR for RFS in the cohort of patients in this study was similar with HR = 0.55 (95% CI: 0.40 to 0.77; p = 0.0003).

STILs data

A comparison was made of the STILs evaluations between the two independent pathologists (SB, HB). There was good concordance of the STILs assessment by deciles with 55% agreement and a weighted Kappa statistic of 0.66 (95% CI: 0.57 to 0.74). The concordance of the dichotomous variable of LPBC status (LPBC if STILs ≥ 60%) classification between the two pathologists was excellent with 98% agreement (Kappa = 0.85, 95% CI: 0.64 to 1.00). The analysis only used the STILs data from the pathologist (H.B.) who reviewed all the specimens.

The distribution of STILs overall and by study arm is presented in Table 1. A majority of the samples were classified as having between 0 to 19% STILs. There were 94 samples (9.9%) that were classified as LPBC and this was balanced between the arms: 48/489 (9.8%) in Arm A and 46/456 (10.1%) in Arm C (p = 0.89).

Characteristics associated with lymphocyte predominant breast cancer

Women with LPBC breast cancer (approximately 10% of all patients) were less likely to have hormone receptor positive (ER+ or PR+ or both) disease compared to women with non-LPBC breast cancer, 31% compared to 57%, respectively (p-value < 0.0001, Table 2). In addition, women with LPBC disease were more likely to have breast conserving surgery (47% versus 38%), to have poor tumor grade (80% versus 70%), and were more likely to be stage N0 (21% versus 13%) although these differences did not achieve statistical significance. There were no other differences in baseline characteristics between the two groups. Importantly, the two groups were balanced with respect to treatment arm assignment.

Recurrence-free survival and STILs

There were 162 disease recurrence events: 8 events in the LPBC group and 154 in the non-LPBC group. Patients without recurrent disease were followed for a median of 6.9 years (min = 0.0 years, max =13.6 years). There was a significant interaction between treatment arm and LPBC status (p = 0.026). In particular, patients with LPBC tumors did not appear to derive any additional benefit from the addition of trastuzumab (HR = 2.43, 95% CI: 0.58 to 10.22; p = 0.22). This is in contrast to patients with non-LPBC tumors who appeared to derive benefit from the addition of trastuzumab (HR = 0.49, 95% CI: 0.35 to 0.69; p-value < 0.0001) to chemotherapy. Figure 2 contains the Kaplan-Meier curves comparing the RFS by

treatment group for each patient group. In Arm A, the 10-year Kaplan-Meier estimates for patients with LPBC tumors and patients with non-LPBC tumors are 90.9% and 64.3%, respectively (p=0.004). The corresponding Kaplan-Meier estimates of 10 year RFS for the two groups were 80.0% and 79.6%, respectively, for patients in Arm C (p=0.79). We performed an exploratory analysis of the ≥50% cut-point and the results are the same. There was limited power in the analysis of LPBC status due to the fact that there are only 8 recurrence events in the LPBC set. Exploratory splitting of the LPBC group into HR-positive and HR-negative groups results in even less power. The number of patients and number of events for the four different groups as defined by LPBC status and HR status are: LPBC and HR-positive: N=29, events=0; LPBC and HR-negative: N=65, events=8; Non-LPBC and HR-positive: N=482, events=84; Non-LPBC and HR-negative: N=369, events=70. In the LPBC and HR-positive group, there are no recurrence events out of 29 patients. This means that in the model it is not possible to get an estimate for the HR-positive in this group (eTable2). However, the relationship in this group appears to be similar to that in the HR-negative group (i.e. both hazard ratios are below 1).

When we adjusted the Cox model for important prognostic variables (age, nodal status, HR status, tumor grade, and tumor size), the interaction term for treatment arm and LPBC status remained significant (p = 0.04) and so we performed separate multivariable analysis for each arm (Table 3; eFigure1). LPBC status was significantly associated with RFS in Arm A (HR = 0.19; 95% CI: 0.06 to 0.61, p-value =0.005) but not in Arm C (HR = 1.01; 95% CI: 0.39 to 2.60; p-value = 0.98). Hormone receptor status was associated with RFS in Arm A (HR = 0.63; 95% CI: 0.42 to 0.94; p-value = 0.02) but not in Arm C (HR = 0.75; 95% CI: 0.43 to 1.32; p-value = 0.32).

When we performed the analysis treated the STILs decile levels as a continuous variable, the relationships observed above did not change. In particular STILs as deciles was associated with RFS in the multivariable model for Arm A (p=0.0002), was not associated with RFS in the multivariable model for Arm C (p=0.84), and the arm by STIL decile interaction was significant (p=0.0082) (eFigure2).

DISCUSSION

In this prospectively defined, retrospective study, we report that STILs assessed dichotomously (LPBC; ≥60% STILs) were significantly associated with outcome in HER2 positive patients treated with chemotherapy alone, i.e., patients with LPBC had a better prognosis following treatment with AC→T without trastuzumab. This association was not observed following treatment with AC→T + trastuzumab. Notably, we did not confirm that increased STILs, either assessed in deciles or dichotomously (LPBC; ≥60% STILs) were predictive of increased benefit from adjuvant trastuzumab. To the contrary, in exploratory analyses from this landmark adjuvant trastuzumab trial, we showed that patients with high STILs (LPBC; ≥60% STILs) did not benefit from the addition of trastuzumab. However, it should be noted that only 94 patients were classified as LPBC and there was a total of only eight disease recurrence events. This means that this study was likely underpowered to detect a treatment effect in this group. On the other hand, the interaction p-value was significant, which indicates that the trastuzumab treatment effect does appear to differ by

LPBC status; at the very least, the trastuzumab effect in the LPBC patients appears to be less than that in the non-LPBC patients.

Previous reports have suggested that increasing STILs, either as a semi-continuous variable (deciles) or as a dichotomous variable (LPBC; ≥60% STILs) is prognostic of decreased residual risk following chemotherapy in ER negative breast cancer.[10,14] We confirmed this semi-continuous association between decreasing residual risk as a function of STILs by decile in patients treated with adjuvant chemotherapy alone and the interobserver concordance was good. Using the dichotomous cutoff of ≥60% STILs, the interobserver concordance was excellent, and the previously prognostic association between those with LPBC and a decreased residual risk was confirmed in chemotherapy treated patients alone.

These data have implications with respect to cancer pathogenesis and metastasis. STILs have been a known prognostic factor for greater than 75 years and we have confirmed this finding in patients with HER2 positive breast cancer treated with chemotherapy without trastuzumab in the adjuvant setting[1]. The presence of a tumor-specific immune response may stimulate immune surveillance in these antigenic primary cancers for primary tumor control, possibly as a function of the composition of the T-cell receptor repertoire of STILs.[21,22] These STILs data may seem counterintuitive to other data from whole transcriptome analyses, which identified a cohort of genes that can be assigned to immune function gene ontology terms and which is predictive of long term RFS in trastuzumab-treated patients.[23]

Specifically, our model[23] was derived from HER2+ samples from a large randomized adjuvant trial of chemotherapy ±trastuzumab in which a large number of unselected genes were assessed and systematically we excluded any genes that were prognostic following chemotherapy. So our model is includes genes that strictly are predictive of trastuzumab response. There are also other important differences between our data and those reported by others. The recently reported Denkert et al study[15] was a neoadjuvant study in which STILs were assessed in smaller needle core biopsy H&E tissue sections, the study included triple negative breast carcinomas which may have higher levels of STILs, the end-point was different, pCR, and the HER2 targeted treatment was different: the inclusion of a TKI in the trial may significantly alter the association between the immune system and the tumor[15]. Importantly, with respect to their immune signature, Denkert et al used a selected candidate genes list, with little or no information provided on how the genes were selected. If the genes were pre-selected on the basis of association with increased lymphocyte infiltration, then their reported association is predictable. Finally, some immune function genes may be expressed in epithelial cells, and the expression of many immune function genes reflects cellular activity, rather than cell number; thus, the relationship between gene expression profiles, derived from mixed cell populations, and number of lymphocytes is complex.

How do these results and other studies of STILs impact clinical treatment decisions, in particular the adjuvant trastuzumab therapy treatment decision? These results do not confirm those of Loi et al.[14] Our findings do not show that increased STILs, either assessed in deciles or dichotomously (LPBC; ≥60% STILs) were predictive of increased benefit from adjuvant trastuzumab; but, that patients with high STILs (LPBC; ≥60% STILs) did not

benefit from the addition of trastuzumab. The observed lack of trastuzumab benefit in the small population of LPBC patients treated with chemotherapy + trastuzumab is limited by the small numbers of patients, limited events and the exploratory nature of the study and they should only be considered hypothesis generating and will require further study in the other landmark adjuvant trastuzumab trials[24,25].

The strengths of this study include: the predefined methods and cutpoints for STILs assessment using consensus guidelines, tandem STILs assessment and adjudication of challenging cases, low degree of interobserver variability in STILs assessment, and study in a landmark adjuvant trastuzumab randomized clinical trial.[20] The major limitation of the study was that only a subset of the enrolled patients on N9831 were included in this analysis although there did not appear to be substantially meaningful difference between patients who provided tissue for analysis and had sufficient tissue for analysis than those who did not.

This was an exploratory analysis of the association between STILs and RFS from a subset of N9831 women with HER2-positive disease treated with chemotherapy alone or treated with concurrent chemotherapy and trastuzumab followed by trastuzumab. These results show that patients with tumors classified as LPBC had better RFS when treated with chemotherapy alone than patients with tumors not classified as having LPBC. Importantly, LPBC status was not associated with RFS in patients treated concurrently with chemotherapy and trastuzumab. A significant treatment interaction between LPBC status and trastuzumab benefit was observed which raises the question whether women with HER2-positive breast cancer with LPBC require treatment with trastuzumab; however, this finding, contradictory to previously published results, will require further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Drs. Baehner and Perez were involved in the conception and design of the manuscript. Acquisition of data was provided by Drs. Perez, Ballman, and Tenner. All authors contributed to the analysis and interpretation of data as well as drafting and final approval of the manuscript, and all authors agree to be accountable for all aspects of the work. We thank Natasha Calhoun for her expert secretarial assistance, all patients who participated in N9831 and provided consent for these studies, as well as collaborators who made this study possible.

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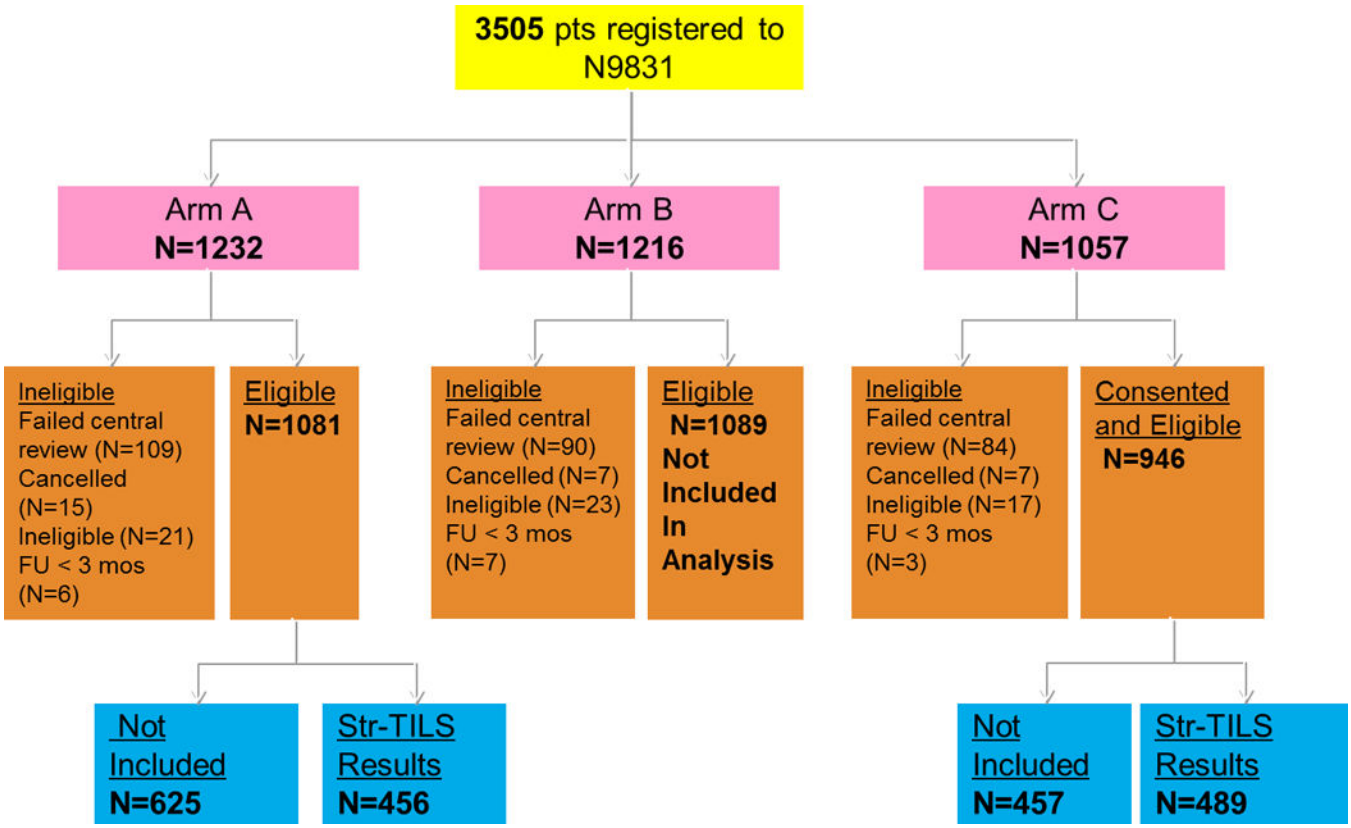

Figure 1. Cohort Patient Flow

An indication of the patients from the patient study who were included in the STILs analysis.

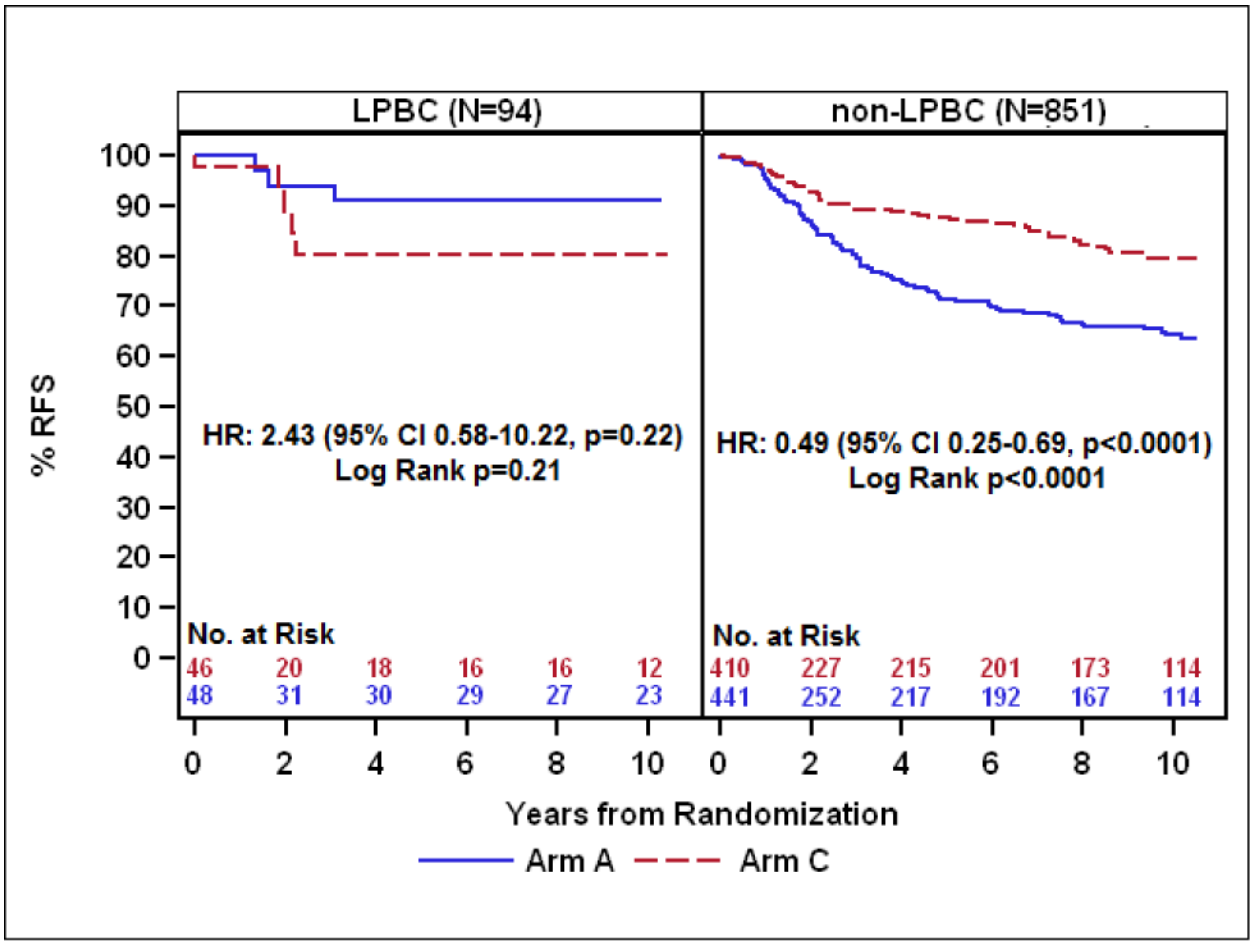

Figure 2. Recurrence-Free Survival by Treatment Arm for patients stratified by STILs Status

A. RFS by Arm, STIL=LPBC. B. RFS by Arm, STIL=non-LPBC. LPBC: lymphocyte predominant breast cancer; Arm A: doxorubicin/cyclophosphamide followed by weekly paclitaxel; Arm C: doxorubicin/cyclophosphamide followed by weekly paclitaxel plus trastuzumab followed by trastuzumab alone.

Table 1

Distribution of STIL classification for each arm separately and for entire group

	Total (N=945)	Arm A (N=489)	Arm C (N=456)	p value
STIL decile, n (%)				0.2899
0–9%	318 (33.7%)	171 (35.0%)	147 (32.2%)	
10–19%	236 (25.0%)	126 (25.8%)	110 (24.1%)	
20–29%	139 (14.7%)	65 (13.3%)	74 (16.2%)	
30–39%	69 (7.3%)	27 (5.5%)	42 (9.2%)	
40–49%	45 (4.8%)	28 (5.7%)	17 (3.7%)	
50–59%	44 (4.7%)	24 (4.9%)	20 (4.4%)	
60–69%	39 (4.1%)	17 (3.5%)	22 (4.8%)	
70–79%	29 (3.1%)	17 (3.5%)	12 (2.6%)	
80–89%	17 (1.8%)	10 (2.0%)	7 (1.5%)	
90–100%	9 (1.0%)	4 (0.8)	5 (1.1%)	
STILs status, n (%)				0.89
LPBC: ≥ 60% STIL	94 (9.9%)	48 (9.8%)	46 (10.1%)	
Non-LPBC: < 60% STIL	851 (90.1%)	441 (90.2%))	410 (89.9%)	

Table 2

Comparison of baseline patient and disease characteristics between patients with LP breast cancer and patients with non-LPBC breast cancer.

Characteristic	LPBC (N=94)	Non-LPBC (N=851)	Total (N=945)	p value
Arm				0.89
A	48 (51%)	441 (52%)	489 (52%)	
C	46 (49%)	410 (48%)	456 (48%)	
Age, years				0.33
mean (SD)	51.0 (11.0)	49.7 (10.3)	49.8 (10.4)	
median (min, max)	50.0 (28.0, 77.0)	49.0 (23.0, 80.0)	50.0 (23.0, 80.0)	
Race, n (%)				0.38
White	85 (90%)	743 (87%)	828 (88%)	
Other	9 (10%)	108 (13%)	117 (12%)	
Menopausal status, n (%)				0.54
pre-menopausal or <50	47 (50%)	454 (53%)	501 (53%)	
post-menopausal or ≥ 50	47 (50%)	397 (47%)	444 (47%)	
Hormone receptor status, n (%)				<0.0001
ER pos and/or PR pos	29 (31%)	482 (57%)	511 (54%)	
ER and PR neg	65 (69%)	369 (43%)	434 (46%)	
Breast surgery, n (%)				0.10
Breast conserving	44 (47%)	324 (38%)	368 (39%)	
Mastectomy	50 (53%)	527 (62%)	577 (61%)	
Nodal status, n (%)				0.27
node positive (1–3+)	30 (32%)	340 (40%)	370 (39%)	
node positive (4–9+)	25 (27%)	217 (25%)	242 (26%)	
node positive (10+)	14 (15%)	120 (14%)	134 (14%)	
positive sentinel node	5 (5%)	64 (8%)	69 (7%)	
Negative sentinel node	11 (12%)	63 (7%)	74 (8%)	
node negative (no pos. nodes)	9 (10%)	47 (6%)	56 (6%)	
Predominant tumor hist, n (%)				0.18
ductal	92 (98%)	805 (95%)	897 (95%)	
other	2 (2%)	45 (5%)	47 (5%)	
missing	0	1	1	
Hist tumor grade (Elson), n (%)				0.06
poor	75 (80%)	590 (70%)	665 (71%)	
well/intermediate	19 (20%)	248 (30%)	267 (29%)	
missing	0	13	13	
Pathologic tumor size, n (%)				0.57

Characteristic	LPBC (N=94)	Non-LPBC (N=851)	Total (N=945)	p value
≤ 2.0 cm	40 (43%)	324 (38%)	364 (39%)	
2.1–5.0 cm	48 (51%)	451 (53%)	499 (53%)	
>5.0 cm	6 (6%)	76 (9%)	82 (9%)	
Tumor stage, n (%)				0.55
1	40 (43%)	324 (38%)	364 (39%)	
2	48 (51%)	450 (53%)	498 (53%)	
3	6 (6%)	77 (9%)	83 (9%)	
N stage, n (%)				0.11
0	20 (21%)	110 (13%)	130 (14%)	
1	67 (71%)	692 (81%)	759 (80%)	
2	7 (7%)	47 (6%)	54 (6%)	
3	0 (0%)	2 (0%)	2 (0%)	
Received hormonal treatment				<0.0001
yes	24 (26%)	462 (55%)	486 (52%)	
no	70 (74%)	384 (45%)	454 (48%)	
missing	0	5	5	

Table 3

Multivariable models for RFS by each arm separately.

	Arm A N = 489 Number of RFS events = 108		Arm C N = 456 Number of RFS events = 54	
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
STILs dichotomous groups				
Age, years	0.98 (0.96–1.00)	0.81	1.00 (0.98–1.03)	0.81
Nodal status				
negative	1.00 (reference)	<0.0001	1.00 (reference)	0.005
1–3 positive	0.70 (0.30–1.62)		3.75 (0.50–28.21)	
4–9 positive	1.50 (0.65–3.43)		3.77 (0.48–29.35)	
10+ positive	2.46 (1.06–5.74)		10.04 (1.31–77.05)	
HR status				
negative ER and negative PR	1.00 (reference)	0.02	1.00 (reference)	0.32
ER and/or PR positive	0.63 (0.42–0.94)		0.75 (0.43–1.32)	
Tumor grade (Elson/SBR)				
poor	1.00 (reference)	0.38	1.00 (reference)	0.37
well/intermediate	0.81 (0.51–1.30)		0.74 (0.39–1.42)	
Pathology tumor size, cm	1.08 (0.98–1.19)	0.12	1.00 (0.94–1.06)	0.98
STIL status				
non-LPBC	1.00 (reference)	0.005	1.00 (reference)	0.98
LPBC	0.19 (0.06–0.61)		1.01 (0.39–2.60)	
STILs quasi-continuous				
Age, years	0.99 (0.97–1.00)	0.13	1.00 (0.98–1.03)	0.82
Nodal status				
negative	1.00 (reference)	<0.0001	1.00 (reference)	0.005
1–3 positive	0.64 (0.28–1.48)		3.79 (0.50–28.44)	
4–9 positive	1.42 (0.62–3.25)		3.78 (0.49–29.46)	
10+ positive	2.33 (1.00–5.42)		10.10 (1.31–77.65)	
HR status				
negative ER and negative PR	1.00 (reference)	0.02	1.00 (reference)	0.32
ER and/or PR positive	0.62 (0.41–0.92)		0.75 (0.43–1.32)	
Tumor grade (Elson/SBR)				
poor	1.00 (reference)	0.15	1.00 (reference)	0.38
well/intermediate	0.70 (0.44–1.13)		0.75 (0.39–1.43)	

	Arm A N = 489 Number of RFS events = 108		**Arm C N = 456 Number of RFS events = 54**	
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Pathology tumor size, cm	1.08 (0.98–1.19)	0.13	1.00 (0.94–1.06)	0.99
STIL	0.79 (0.70–0.89)	0.0002	1.01 (0.89–1.15)	0.85