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ORIGINAL REPORT

Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Recent studies suggest that tumor-infiltrating lymphocytes (TILs) are associated with disease-free (DFS) and overall survival (OS) in operable triple-negative breast cancer (TNBC). We seek to validate the prognostic impact of TILs in primary TNBCs in two adjuvant phase III trials conducted by the Eastern Cooperative Oncology Group (ECOG).

Patients and Methods
Full-face hematoxylin and eosin–stained sections of 506 tumors from ECOG trials E2197 and E1199 were evaluated for density of TILs in intraepithelial (iTILs) and stromal compartments (sTILs). Patient cases of TNBC from E2197 and E1199 were randomly selected based on availability of sections. For the primary end point of DFS, association with TIL scores was determined by fitting proportional hazards models stratified on study. Secondary end points were OS and distant recurrence–free interval (DRFI). Reporting recommendations for tumor marker prognostic studies criteria were followed, and all analyses were prespecified.

Results
The majority of 481 evaluable cancers had TILs (sTILs, 80%; iTILs, 15%). With a median follow-up of 10.6 years, higher sTIL scores were associated with better prognosis; for every 10% increase in sTILs, a 14% reduction of risk of recurrence or death ($P = .02$), 18% reduction of risk of distant recurrence ($P = .04$), and 19% reduction of risk of death ($P = .01$) were observed. Multivariable analysis confirmed sTILs to be an independent prognostic marker of DFS, DRFI, and OS.

Conclusion
In two national randomized clinical trials using contemporary adjuvant chemotherapy, we confirm that stromal lymphocytic infiltration constitutes a robust prognostic factor in TNBCs. Studies assessing outcomes and therapeutic efficacies should consider stratification for this parameter.

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INTRODUCTION

Evidence suggests that the immune system influences breast cancer prognosis.[1] Lymphocytic infiltrates in breast cancer were described decades ago; they are most prominent in aggressive tumors and linked to outcome in these subtypes.[2] More recently, tumor-infiltrating lymphocytes (TILs) have been evaluated in randomized trials using contemporary chemotherapy; these studies have confirmed that TILs are most frequently found in highly proliferative tumors (triple-negative [TNBC] and human epidermal growth factor receptor 2 [HER2] –positive breast cancers) and that their presence at diagnosis is associated with pathologic response to neoadjuvant therapy as well as disease-free (DFS) and overall survival (OS) after adjuvant chemotherapy in certain subtypes.[3-5] Specifically, Loi et al[4] evaluated the relationship between TILs at diagnosis with clinical outcome in the BIG 02-98 adjuvant phase III trial and showed a significant association in TNBC. If confirmed in an independent study, this would suggest that routine assessment and quantification of TILs could provide clinically meaningful prognostic information in TNBC.

We herein describe a prospective-retrospective validation study performed in accordance with guidelines recommended by Simon et al[6] and the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria.[7] The purpose of our study was to provide confirmatory evidence of clinical validity for TILs as a prognostic biomarker in TNBC. We used archived samples from two adjuvant phase III trials coordinated by the Eastern Cooperative Group (ECOG) in collaboration with the North American Breast Cancer Groups and evaluated the prognostic utility of TILs within intraepithelial (iTILs) and stromal compartments (sTILs) in TNBCs treated with adjuvant anthracycline-containing chemotherapy.

PATIENTS AND METHODS

Study Patients

TIL analysis was retrospectively performed on prospectively collected formalin-fixed paraffin-embedded hematoxylin and eosin (HE) –stained sections from two ECOG-sponsored randomized, prospective phase III adjuvant trials (E2197 and E1199). All samples were collected at baseline from the surgical specimens. Patients enrolled onto both trials had consented to use of their tumor tissue for research purposes, and this study was approved by the Breast Cancer Intergroup of North America (TBCI) committee. In addition, institutional review board approval was obtained from Indiana University.

In E2197, 2,952 women with T1c to T3N0 or T1-3N1 breast cancer were enrolled between July 1998 and January 2000 and randomly assigned to doxorubicin (60 mg/m^2) plus either cyclophosphamide (600 mg/m^2) or docetaxel (60 mg/m^2) once every 3 weeks for four cycles. For this analysis, we used a subset of 776 tumors that had central evaluation of histologic grade and estrogen receptor (ER), progesterone receptor (PR), and HER2 by immunohistochemistry on tissue microarrays as previously described[8]; of these, 250 samples were TNBCs, and 191 of these still had slides available for review (Appendix Fig A1, online only). ER and PR were considered negative using the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (< 1%).[9]

In E1199, 5,052 women with T1-3N1-2 or T2-3N0 breast cancer were enrolled between October 1999 and January 2002 and randomly assigned to

Table 1. Characteristics of Patient Subsets Included in Analysis and Corresponding Population for Each Study

Characteristic	E1199 (n = 291)		E2197 (n = 190)		Total (N = 481)		All TNBCs in E1199 (n = 926)*		All Hormone Receptor–Negative BCs in E2197 (n = 922)†	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age, years										
24-40	66	22.7	38	20.0	104	21.6	198	21.4	177	19.2
41-50	108	37.1	68	35.8	176	36.6	335	36.2	330	35.8
51-60	77	26.5	60	31.6	137	28.5	260	28.1	280	30.4
61-85	40	13.7	24	12.6	64	13.3	133	14.4	135	14.6
Menopausal status										
Premenopausal	164	56.4	95	50.0	259	53.8	489	52.8	466	50.5
Postmenopausal	127	43.6	95	50.0	222	46.2	437	47.2	456	49.5
No. of lymph nodes										
0	56	19.2	141	74.2	197	41.0	182	19.7	717	77.8
1-3	150	51.5	49	25.8	199	41.4	484	52.4	205	22.2
> 3	85	29.2	0	0.0	85	17.7	258	27.9	0	0.0
Tumor size, cm										
0.1-2.0	75	25.8	82	43.2	157	32.6	243	26.3	416	45.1
2.1-5.0	184	63.2	99	52.1	283	58.8	586	63.4	466	50.5
> 5.0	32	11.0	9	4.7	41	8.5	95	10.3	40	4.3
Receptor status										
Triple negative	291	100.0	190	100.0	481	100.0	926	100.0	NA†	
Primary surgery										
Local excision	137	47.1	114	60.0	251	52.2	408	44.1	520	56.4
Mastectomy	154	52.9	76	40.0	230	47.8	517	55.9	402	43.6
Race										
White	243	83.5	160	84.2	403	83.8	728	78.6	776	84.3
Black	33	11.3	26	13.7	59	12.3	137	14.8	106	11.5
Other	11	3.8	3	1.6	14	2.9	52	5.6	28	3.0
Unknown	4	1.4	1	0.5	5	1.0	9	1.0	12	1.3
Treatment										
Four cycles of AT	0	0.0	87	45.8	87	18.1	0	0.0	461	50.0
Four cycles of AC (without sequential paclitaxel)	0	0.0	103	54.2	103	21.4	0	0.0	461	50.0
AC → paclitaxel every 3 weeks	73	25.1	0	0.0	73	15.2	237	25.6	0	0.0
AC → paclitaxel once per week	86	29.6	0	0.0	86	17.9	246	26.6	0	0.0
AC → docetaxel every 3 weeks	66	22.7	0	0.0	66	13.7	228	24.6	0	0.0
AC → docetaxel once per week	66	22.7	0	0.0	66	13.7	215	23.2	0	0.0

Abbreviations: AC, doxorubicin plus cyclophosphamide; AT, doxorubicin plus paclitaxel; BC, breast cancer; HER2, human epidermal growth factor receptor 2; NA, not available; TNBC, triple-negative breast cancer.

*Lymph node status and tumor size were unknown for two patient cases; type of surgery was unknown for one patient case.

†HER2 status not available for full cohort; data presented for hormone receptor–negative subgroup.

one of four taxane regimens after doxorubicin plus cyclophosphamide once every 3 weeks for four cycles: taxol 175 mg/m^2 once every 3 weeks for four cycles, taxol 80 mg/m^2 once per week for 12 weeks, docetaxel 100 mg/m^2 once every 3 weeks for four cycles, or docetaxel 35/m^2 once per week for 12 weeks.[10] Of 926 patients with TNBC enrolled onto E1199, based on local institution determination of ER, PR, and HER2, 315 were randomly selected for review as guided by the prespecified power analysis (Appendix Fig A1, online only). For this subset, ER and PR negativity was defined by local laboratories before adoption of the ASCO/CAP guidelines and was defined as $< 10\%$ immunostaining.

Pathologic Assessment

Histopathologic analysis of percentage of TILs was performed on a single full-face HE-stained tumor section using criteria described by Denkert et al[3] and Loi et al.[4] iTILs were defined as the percentage of lymphocytes in direct contact with the tumor cells, whereas sTILs were defined as the percentage of tumor stroma containing infiltrating lymphocytes. Areas of in situ carcinoma and crush artifacts were not included. As proposed by Loi et al, we categorized lymphocyte-predominant breast cancer (LPBC) as that involving $\geq 50\%$ lymphocytic infiltration of either tumor stroma or cell nests.

Histopathologic evaluation of TILs was jointly performed by two breast pathologists (S.B., S.D.), who were blinded to clinical information, including treatment allocation and outcomes. All tumors in the study were jointly evaluated, and the results were reported in increments of 10; 0 was defined as 0% to 1%, and all other estimates were rounded up to the next highest decile (ie, 11% to 20% represents TIL score of 20). In addition, an independent read was performed on a subset of cases to test analytic validity.

Statistical Analyses

The prespecified primary end point for both the E2197 and E1199 trials was DFS, defined as time from date of random assignment to date of first recurrence, second primary malignancy, or death resulting from any cause, whichever occurred first. Patients who were alive and disease free were censored at date of last contact. Secondary end points included OS and distant recurrence–free interval (DRFI), which were defined as time from random assignment to death regardless of cause and distant metastatic recurrence, respectively.

All statistical analyses were performed by one of the authors (R.G.), who serves as head biostatistician for ECOG. For the primary test of DFS, TIL scores were classified as high or low using the LPBC cutoff; groups were compared using Cox proportional hazards regression models stratified on study. Secondary analyses included DRFI and OS as well as comparing TIL scores of 0% versus $> 0\%$ and for TIL scores as continuous variables. Multivariable analysis was conducted with known histopathologic and demographic prognostic factors using proportional hazards models. Because weighted sampling was used for selecting patient cases for central review in E2197, weighted analyses were performed to provide valid estimates of population effects in this cohort; with weights equal to the inverse sampling fractions, standard robust variance estimates were used.[11] This is valid for simple cohort sampling schemes[12] and for weighted Cox model analysis.[13] A weight of 1 was used for all E1199 patient cases (because they were selected at random) and for recurrences in E2197; nonrecurrences in E2197 were given a weight of 1.43. These sampling weights were also used in Kaplan-Meier estimates of distribution.

The analysis plan was developed before the project commenced and received approval by the Breast Cancer Intergroup of North America (TBCI). Power calculations were based on results by Loi et al,[4] who observed 10.5% of TNBCs as LPBCs and estimated the DFS hazard ratio (HR) to be 0.3 (95% CI, 0.11 to 0.81). For validation, we targeted a smaller effect size (HR, 0.4) and estimated that with 500 patient cases, assuming the distribution of TIL scores seen by Loi et al and adjusting for weighted sampling, the study would have 83% power for an HR of 0.4 for LPBC versus non-LPBC. In addition, the analysis of continuous sTIL scores would have 80% power for a 21% reduction in the DFS hazard for each 10% increase in sTILs, for a 17.5% reduction in E1199 and a 14% reduction in the combined group.

To test analytic validity, a random subset of 100 patient cases from the study population was selected, and TILs were independently scored by the pathologists. On the basis of the prespecified analysis plan, interobserver agreement and individual agreement with the consensus read were measured as the proportion with scores within $\pm$ 10 points, with exact binomial CIs. Two-sided P values were used for all analyses. REMARK criteria[7] were followed for this study.

RESULTS

Baseline Clinical Characteristics

Tumor specimens from 506 patients (E2197, n = 191; E1199, n = 315) were evaluated for lymphocytic infiltration. For 25 patient cases, TILs could not be adequately assessed, because of either tissue quality or tumor location within the lymph nodes. Therefore, 481 patient cases were included for analysis (E2197, n = 190; E1199, n = 291); their characteristics are listed in Table 1. There were no significant differences in the examined variables between the subset used for analysis and the corresponding target trial population based on characteristics collected prospectively in the two studies, confirming a representative selection (Table 1).

Analytic Validity

An excellent interobserver correlation was demonstrated in a subset of 100 patient cases (evaluable, n = 99). Rates of agreement within 10 percentage points between the two pathologists were as follows: 85% (95% CI, 76% to 91%) for sTILs and 97% (95% CI, 91% to 99%) for iTILs. Rates of agreement between pathologist one and the consensus read were 90% (95% CI, 82% to 95%) for sTILs and 97% (95% CI, 91% to 99%) for iTILs. Rates of agreement between pathologist two and the consensus read were 87% (95% CI, 79% to 93%) for sTILs and 94% (95% CI, 87% to 98%) for iTILs. If category cut points from the Kaplan-Meier analysis are used (not prespecified), the κ statistic showed moderate agreement between the two pathologists (sTILs, 0.40; iTILs, 0.43).

TIL Distribution and Association With Clinicopathologic Variables

Lymphocytic infiltration was observed in the majority of tumors but was significantly greater in stromal compared with intraepithelial

Table 2. Distribution of TIL Scores

	Cancers With iTILs				Cancers With sTILs			
	E1199 (n = 291)	E2197 (n = 190)	Total (n = 481)		E1199 (n = 291)	E2197 (n = 190)	Total (n = 481)	
Score	No.	No.	No.	%	No.	No.	No.	%
0	242	169	411	85	45	50	95	20
10	40	17	57	12	146	91	237	49
20	6	3	9	2	46	36	82	17
30	2	1	3	< 1	26	11	37	8
40	0	0	0	0	9	0	9	2
50	1	0	1	< 1	8	1	9	2
60	0	0	0	0	4	0	4	< 1
70	0	0	0	0	4	0	4	< 1
80	0	0	0	0	3	1	4	< 1
90	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

NOTE. No. of patient cases within intratumoral and stromal compartments by study and combined with percentages of tumors.

Abbreviations: iTIL, intraepithelial tumor-infiltrating lymphocyte; sTIL, stromal tumor-infiltrating lymphocyte; TIL, tumor-infiltrating lymphocyte.

areas (Table 2); 80% of tumors had at least 10% sTILs (range, 10% to 80%), but only 15% of cancers had at least 10% iTILs (range, 10% to 50%). In the whole population, the median percentage of iTILs was 0% (interquartile range, 0% to 0%), which was lower than that for sTILs (10%; interquartile range, 10% to 20%). Examples of varying degrees of infiltration are shown in Figure 1. The correlation between mean percentage sTIL and iTIL assessments was 0.47 ($P < .001$), with one exception; patient cases with iTILs always had stromal infiltrates.

LPBC ($\geq$ 50% TILs; Fig 1) was seen in only 4.4% of TNBCs (21 of 481). Germinal centers were noted in a small fraction of cancers ($<$ 1%) and not included in the analysis.

There were no strong associations between TIL scores and other factors examined (age, menopausal status, or tumor size), with the exception of lymph node status. The likelihood of an sTIL score of 0 decreased as the number of positive nodes increased, with a rate of 25.4% (50 of 197) for node-negative breast cancers, 18.1% (36 of 199)

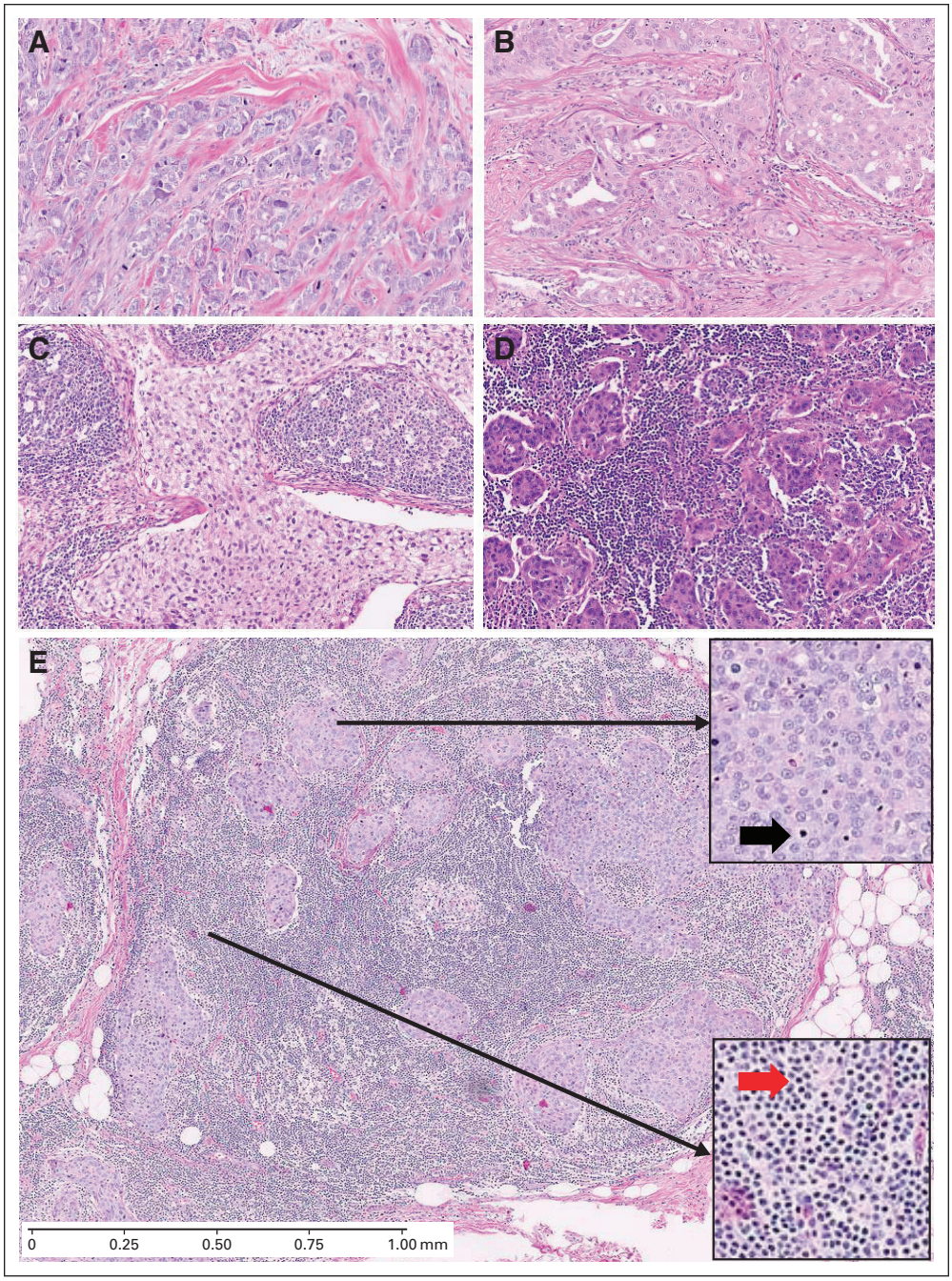

Fig 1. Variable degree of lymphocytic infiltrate in triple-negative breast cancer on hematoxylin and eosin (HE) –stained tumor sections. Examples of tumors without intraepithelial tumor-infiltrating lymphocytes (TILs) but varying degrees of stromal TILs by HE: (A) 0%, (B) 20%, (C) formation of germinal follicles, and (D) 80% (×10 magnification). (E) Representative example of lymphocyte-predominant breast cancer (×5 magnification) with 10% intraepithelial TILs (inset, black arrow, lymphocytes in direct contact with cancer cells) and 80% stromal TILs (inset, red arrow, abundant lymphocytes within stroma).

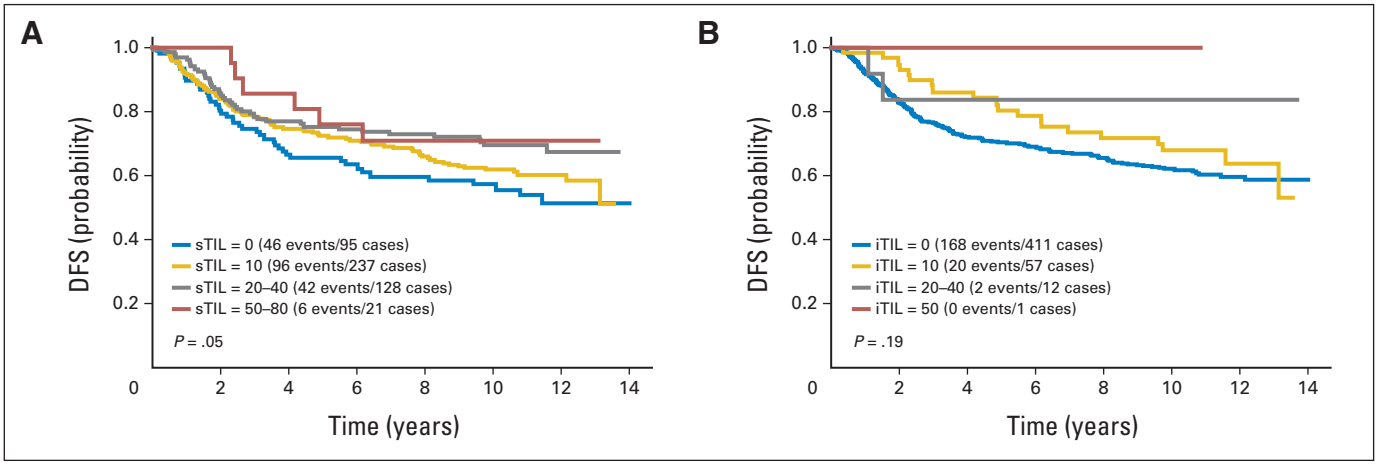

Fig 2. Prognostic value of tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer. Kaplan-Meier curves of estimated disease-free survival (DFS) for all patients for (A) stromal TIL (sTIL) score and (B) intraepithelial TIL (iTIL) score (grouped as 0 [defined as 0% to 1%] *v* 10 [2% to 10%] *v* 20 to 40 [11% to 40%] *v* 50 [41% to 50%] or *v* 50 to 80 [41% to 80%]); *P* values are for comparison of four groups.

for tumors with one to three positive nodes, and 10.6% (nine of 85) for tumors with > three nodes (χ^2 $P = .01$).

Association of TILs With DFS

For the prognostic evaluations, all treatment arms were pooled. Among the 481 patient cases, there were 190 DFS events (E1199, 118 events; E2197, 72 events), with 133 of these being recurrences (E1199, 87; E2197, 46; other events were new primary cancers and deaths without prior recurrence). The median follow-up was 10.6 years (E1199, 10.1 years; E2197, 12.6 years).

Continuous TIL scores were associated with DFS in univariable analysis (sTILs, $P = .02$; iTILs, $P = .06$), with estimated HRs for a 10-point change in sTIL and iTIL scores being 0.86 (95% CI, 0.76 to 0.98; $P = .02$) and 0.72 (95% CI, 0.51 to 1.02; $P = .06$), respectively (Fig 2). Because of the association of sTIL score with lymph node involvement, which is a known prognostic factor for DFS, the sTIL score was more significant when nodes were taken into account (sTILs: HR, 0.82; 95% CI, 0.72 to 0.94; $P = .003$; iTILs: HR, 0.73; 95% CI, 0.52 to 1.02; $P = .07$). The intraepithelial score did not reach significance, because most patient cases had scores of 0, even though the estimated effect was larger. Although the analysis was only adequately powered for the combined analysis for E2197 and E1199, data are provided separately by study in Appendix Table A1 (online only).

When TILs were assessed as a binary variable (> 0 *v* 0), the estimated HR was 0.69 (95% CI, 0.49 to 0.98; $P = .04$) for presence of sTILs compared with the absence of sTILs and 0.69 (95% CI, 0.45 to 1.06; $P = .09$) for the presence of iTILs versus the absence of iTILs. The binary analysis for LPBC had limited power, with only 21 patient cases (4.4%). The estimated HR for LPBC was 0.59 (95% CI, 0.27 to 1.28; $P = .18$) in univariable analysis and 0.45 (95% CI, 0.19 to 1.03; $P = .06$) in multivariable analysis including nodal involvement.

Association of TILs With DRFI and OS

Among the 481 patient cases, 107 events of distant recurrence and 142 deaths were reported. sTIL score was significantly correlated with DRFI and OS. The estimated HR for a 10-point change in sTIL score (estimated from linear term without adjustment for other factors) was 0.82 (95% CI, 0.68 to 0.99; $P = .04$) for DRFI and 0.81 (95%

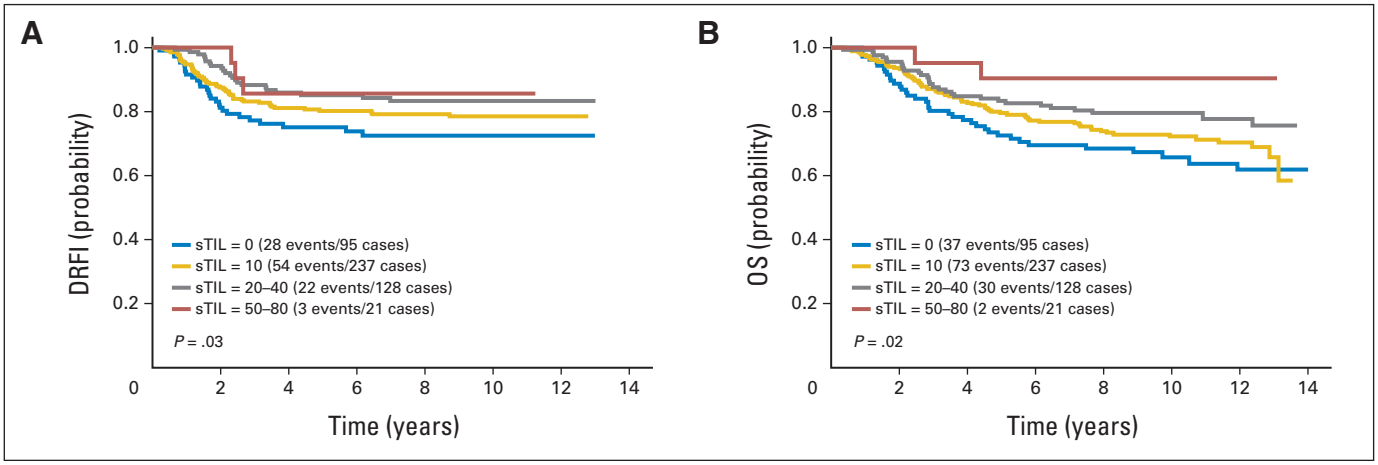

Fig 3. Prognostic value of stromal tumor-infiltrating lymphocytes (sTILs) in triple-negative breast cancer. Kaplan-Meier curves of estimated (A) distant recurrence–free interval (DRFI) and (B) overall survival (OS) for all patients for sTILs (grouped as 0 [defined as 0% to 1%] *v* 10 [2% to 10%] *v* 20 to 40 [11% to 40%] *v* 50 to 80 [41% to 80%]); *P* values are for comparison of four groups.

Table 3. Estimated DFS, DRFI, and OS in Multivariable Model for Linear Effect of Stromal TIL Score

Variable	DFS			DRFI			OS		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Stromal TIL (10% increase)	0.84	0.74 to 0.95	.005	0.81	0.68 to 0.97	.02	0.79	0.67 to 0.92	.003
Tumor size ≤ 2.0 *v* 2.1-5.0 cm	0.58	0.41 to 0.81	.002	0.51	0.31 to 0.84	.008	0.52	0.34 to 0.79	.002
Tumor size ≤ 2.0 *v* > 5.0 cm	0.47	0.27 to 0.81	.007	0.31	0.16 to 0.61	< .001	0.41	0.22 to 0.75	.004
Node negative *v* 1-3 lymph nodes	0.53	0.36 to 0.78	.001	0.48	0.28 to 0.82	.007	0.48	0.30 to 0.76	.002
Node negative *v* > 3 lymph nodes	0.23	0.14 to 0.38	< .001	0.20	0.11 to 0.39	< .001	0.17	0.10 to 0.31	< .001
Age 41-50 *v* 24-40 years	1.30	0.83 to 2.10	.24	1.90	0.96 to 3.70	.06	1.50	0.85 to 2.80	.15
Age 51-60 *v* 24-40 years	1.40	0.88 to 2.30	.15	2.30	1.20 to 4.60	.02	1.80	1.00 to 3.30	.05
Age > 60 *v* 24-40 years	2.20	1.30 to 3.60	.003	1.90	0.88 to 4.30	.10	2.90	1.60 to 5.30	< .001

Abbreviations: DFS, disease-free survival; DRFI, distant recurrence–free interval; HR, hazard ratio; OS, overall survival; TIL, tumor-infiltrating lymphocyte.

CI, 0.69 to 0.95; $P = .01$) for OS (Fig 3). The intraepithelial score correlated with both DRFI and OS but did not reach significance (DRFI: HR, 0.53; 95% CI, 0.25 to 1.09; $P = .08$; OS: HR, 0.64; 95% CI, 0.39 to 1.05; $P = .08$).

Multivariable Analysis

Multivariable analysis including prognostic variables obtained prospectively in both trials (tumor size, lymph node status, and age) confirmed sTILs to be an independent prognostic factor for DFS, DRFI, and OS (Table 3). Grade was not included in the multivariable analysis, because it was not prospectively collected in E1199, and grade is less relevant for this subtype because most TNBCs are high grade. However, when grade was included for the E2197 stratum, the general results of the multivariable analysis did not change (data not shown).

DISCUSSION

In this study, we confirmed that stromal lymphocytic infiltration constitutes a robust and independent prognostic marker in TNBCs treated with adjuvant chemotherapy, with increasing sTILs predictive of a significantly lower risk of recurrence or death, distant recurrence, and overall mortality. We analyzed 481 TNBC samples prospectively collected in two phase III adjuvant randomized breast cancer trials in the United States. At a median follow-up of 10.6 years, sTILs as a continuous variable were associated with DFS, with an estimated HR of 0.84 ($P = .005$) for a 10-point change in sTIL score. Associations for iTILs and LPBC with outcome were also observed but did not reach statistical significance, because only a small percentage of cancers displayed these. Importantly, the prognostic significance of sTILs was independent of known prognostic factors, and in our population of node-positive and node-negative cancers, it improved outcome prediction even more significantly when lymph node status was taken into account. DRFI and OS, which are undoubtedly the most important outcome measures for patients with TNBC, also showed a significant association with sTILs (DRFI: HR, 0.82; $P = .04$; OS: HR, 0.81; $P = .01$) for each 10-point increase in sTILs.

Lymphocytic infiltration in primary breast cancers was described decades ago, often in the context of medullary breast cancers,[14] and some observations suggested prognostic value.[2-5,15,16] However, only few studies used patient cases from controlled randomized trials, including the adjuvant series by Loi et al[4] and the neoadjuvant studies by Denkert et al[3] and West et al.[5] Specifically, Loi et al showed that TILs are more frequent in TNBCs compared with hormone receptor–positive breast cancers and associated with outcome in TNBCs in the BIG 02-98 phase III trial.[4] To reach level I evidence for biomarkers, results based on retrospective analysis using archived tissues from a randomized trial (category B study) must be confirmed in an independent randomized trial that has been designed, conducted, and analyzed in a similar manner, and the results must be equally compelling (Simon et al[6]). In our confirmatory study, we used archived tissue specimens from independent, high-quality US phase III adjuvant trials with long-term follow-up and prospectively evaluated the clinical validity of TILs. Full-face HE-stained sections were examined, and all analyses were prespecified. In concordance with Loi et al, sTILs as a continuous variable were associated with a better prognosis in TNBC. The effect size noted by the two studies was remarkably similar, with each 10% increase in sTILs being associated with a 15% reduced risk of relapse or death ($P = .025$) and 17% reduced risk of death ($P = .023$) in the Loi study and a 14% reduced risk of relapse or death ($P = .02$) and 19% reduced risk of death ($P = .01$) in our study. Because OS point estimates were numerically better than DFS point estimates in both studies, it should be further studied whether the ability to generate an antitumor immune response also has general health benefits.

To date, the strongest association of TILs and breast cancer outcome has been shown for TNBCs,[4,15] which are poorly differentiated tumors and therefore may contain more antigenic tumor variants compared with other HER2-negative breast cancer subtypes. In fact, cancer testis antigens such as MAGE-A3 and NY-ESO-1 are most prevalent in TNBCs, as demonstrated by several groups.[17,18] TILs at diagnosis therefore likely indicate an ongoing antitumor immune response, which can contribute to improved outcomes (as shown for other solid tumors[19,20]), although underlying mechanisms such as increased response to cytotoxics (suggested by neoadjuvant study[3]) and/or eradication of micrometastatic disease have not yet been fully investigated.

Of note, all patients in the ECOG studies and BIG 02-98 trial received adjuvant anthracycline-containing chemotherapy. The prognostic utility of TILs in TNBC is therefore limited to patients who undergo adjuvant chemotherapy. Another limitation is the low number of LPBCs, which limited the power to determine its prognostic value. However, the continuous score will likely be most useful in practice because the binary cutoff at 50% was arbitrary and was merely chosen to highlight good outcomes of patients with extensively infiltrated cancers, rather than to imply the existence of a distinct biologic

subgroup.[4] It is important to stress for the nonpathologist that although sTILs are not in direct contact with cancer cells, these TILs are still located within the tumor.

Our study raises several questions for future investigation. Does LPBC correlate with the recently identified immunomodulatory subset of TNBC?[21] Can immunomodulating therapies administered at diagnosis increase TILs and improve clinical outcome in TNBC? Can conventional cancer therapeutics be harnessed for their induction of immunogenic cell death?[22,23] Can further delineation of infiltrating immune-cell subsets (eg, programmed death-1 expression by lymphocytes, which can contribute to prognosis[24]) and chemokine profiles identify additional targets for therapies?

In conclusion, we provide clinical validation that increasing stromal lymphocytic infiltration assessed on full-section HE-stained tumors on a continuous scale is a robust and independent prognostic factor for TNBC. The results of this study as an independent category B data set, together with the data published by Loi et al,[4] provide level I evidence of sTILs as a prognostic indicator in TNBCs treated with adjuvant chemotherapy. This marker should therefore be considered for prospective inclusion in clinical trials and routine histopathologic evaluation of operable TNBCs, and efforts are ongoing to obtain procedural standardization among several laboratories.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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GLOSSARY TERMS

biomarker (biologic marker): a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

cancer-testis antigens: proteins expressed on the surface of cancer and testicular cells capable of eliciting an immune response outside of the immunologically shielded testis.

Cox proportional hazards regression model: a statistical model for regression analysis of censored survival data, examining the relationship of censored survival distribution to one or more covariates. This model produces a baseline survival curve, covariate coefficient estimates with their standard errors, risk ratios, 95% CIs, and significance levels.

HER2/neu (human epidermal growth factor receptor 2): also called ErbB2. HER2/neu belongs to the epidermal growth factor receptor (EGFR) family and is overexpressed in several solid tumors. Like EGFR, it is a tyrosine kinase receptor whose activation leads to proliferative signals within the cells. On activation, the human epidermal growth factor family of receptors are known to form homodimers and heterodimers, each with a distinct signaling activity. Because HER2 is the preferred dimerization partner when heterodimers are formed, it is important for signaling through ligands specific for any members of the family. It is typically overexpressed in several epithelial tumors.

histopathologic: the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histologic sections have been placed onto glass slides. The vast majority of cancer diagnoses are made by pathologists. The medical diagnosis is formulated as a pathology report describing the histologic findings and the opinion of the pathologist. Evaluation of the diagnosis and the prognosis is required for most treatment protocols.

neoadjuvant therapy: the administration of chemotherapy prior to surgery. Induction chemotherapy is generally designed to decrease the size of the tumor prior to resection and to increase the rate of complete (R0) resections.

NY-ESO-1: gene coding for antigens recognized on neoplastically transformed cells T cells; also known as CTAG1B or cancer/testis antigen 1B.

REMARK criteria: guidelines for reporting tumor marker studies, which include a statement of objectives and a description of patient population and treatments received, biologic materials, and assay methods. Criteria also include guidelines for reporting data, results, and discussion.

triple-negative phenotype: breast tumors that are negative for progesterone and estrogen and that underexpress HER2.

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Appendix

Table A1. Estimated DFS in Univariable Model for Linear Effect of TIL Scores by Study

	E1199			E2197		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
Stromal TIL (10% increase)	0.91	0.81 to 1.03	.14	0.71	0.51 to 0.98	.04
Intraepithelial TIL (10% increase)	0.68	0.44 to 1.05	.08	0.81	0.45 to 1.44	.47

Abbreviations: DFS, disease-free survival; HR, hazard ratio; TIL, tumor-infiltrating lymphocyte.

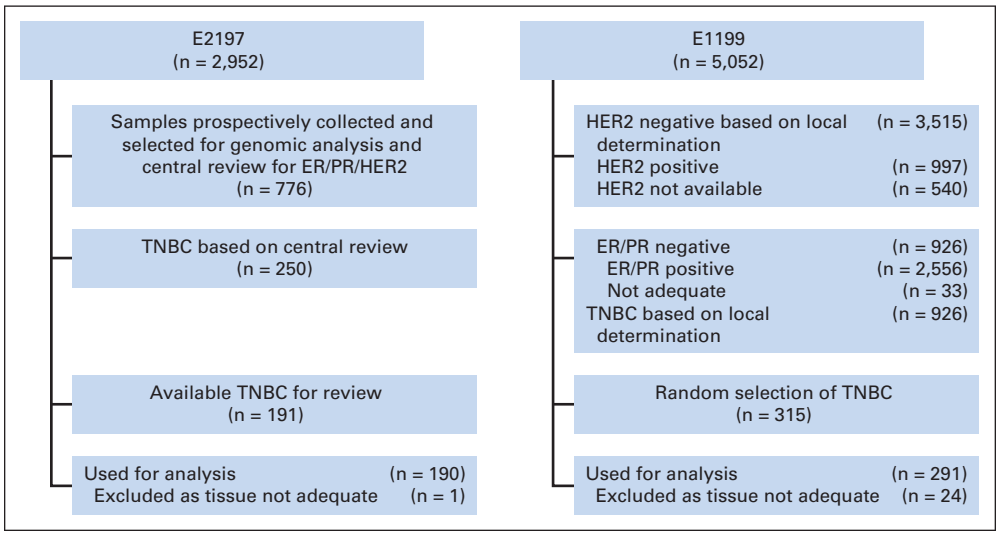

Fig A1. Flow diagram of breast cancer specimens used from E2197 and E1199. To reach sample size of 500, as required by prespecified power analysis, samples were needed from second trial in addition to well-characterized cohort from E2197. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; TNBC, triple-negative breast cancer.