Tumor-Infiltrating Lymphocytes and Prognosis: Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers

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abstract

PURPOSE The aim of the current study was to conduct a pooled analysis of studies that have investigated the prognostic value of tumor-infiltrating lymphocytes (TILs) in early-stage triple negative breast cancer (TNBC).

METHODS Participating studies had evaluated the percentage infiltration of stromally located TILs (sTILs) that were quantified in the same manner in patient diagnostic samples of early-stage TNBC treated with anthracycline-based chemotherapy with or without taxanes. Cox proportional hazards regression models stratified by trial were used for invasive disease-free survival (iDFS; primary end point), distant disease-free survival (D-DFS), and overall survival (OS), fitting sTILs as a continuous variable adjusted for clinicopathologic factors.

RESULTS We collected individual data from 2,148 patients from nine studies. Average age was 50 years (range, 22 to 85 years), and 33% of patients were node negative. The average value of sTILs was 23% (standard deviation, 20%), and 77% of patients had 1% or more sTILs. sTILs were significantly lower with older age ($P = .001$), larger tumor size ($P = .01$), more nodal involvement ($P = .02$), and lower histologic grade ($P = .001$). A total of 736 iDFS and 548 D-DFS events and 533 deaths were observed. In the multivariable model, sTILs added significant independent prognostic information for all end points (likelihood ratio χ^2, 48.9 iDFS; $P < .001$; χ^2, 55.8 D-DFS; $P < .001$; χ^2, 48.5 OS; $P < .001$). Each 10% increment in sTILs corresponded to an iDFS hazard ratio of 0.87 (95% CI, 0.83 to 0.91) for iDFS, 0.83 (95% CI, 0.79 to 0.88) for D-DFS, and 0.84 (95% CI, 0.79 to 0.89) for OS. In node-negative patients with sTILs $\geq$ 30%, 3-year iDFS was 92% (95% CI, 89% to 98%), D-DFS was 97% (95% CI, 95% to 99%), and OS was 99% (95% CI, 97% to 100%).

CONCLUSION This pooled data analysis confirms the strong prognostic role of sTILs in early-stage TNBC and excellent survival of patients with high sTILs after adjuvant chemotherapy and supports the integration of sTILs in a clinicopathologic prognostic model for patients with TNBC. This model can be found at www.tilsinbreastcancer.org.

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INTRODUCTION

The host microenvironment is usually not considered when making prognostic assessment for patients with breast cancer. We and others have previously shown that a higher quantity of immune infiltrate present in both early-stage triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2)–positive breast cancer samples, as quantified using a simple method on standard diagnostic hematoxylin and eosin full section–stained slides, is associated with significantly improved clinical outcomes.[1] This simple method of quantifying tumor-infiltrating lymphocytes (TILs) as the percentage infiltration in the tumor and surrounding stroma has been demonstrated to be a reproducible biomarker that could allow for relatively straightforward implementation in standard clinical pathology globally.[2,3]

Currently, the main prognostic factors in early-stage TNBC represent anatomic tumor burden. In this pooled analysis of patient data obtained mainly from large clinical studies, we sought to establish conclusively the prognostic value of TILs in this breast cancer subtype. Such evidence would strongly support the implementation of a novel biologic biomarker in future breast cancer prognostic staging systems for early-stage TNBC,[4] as a clinical trials stratification factor, and potentially guide immunotherapeutic development in breast cancer. With the success of immune checkpoint blockade in other solid tumor types, clarity on the prognostic role of breast cancer immune infiltrate is now warranted.

The primary objective of the current study was therefore to achieve a robust understanding of the prognostic value of the stromal TILs (sTILs) biomarker

in early-stage TNBC treated with standard adjuvant chemotherapy. We did this by performing a pooled analysis of data that were derived from more than 2,000 patients from multiple individual studies in which TILs had been evaluated in the same manner on standard diagnostic hematoxylin and eosin–stained slides using prespecified criteria.[3] The sTILs variable was chosen a priori to be used in the primary analysis, with intratumoral TILs (iTILs) only in secondary analyses. Large data sets from multiple different types of studies provide new opportunities for the development and validation of prediction models[5]; therefore, our secondary objective was to develop and validate a prognostic model that incorporated the evaluation of TILs with traditional clinicopathologic factors that would help clinicians to provide an integrated and more accurate estimation of prognosis. We also sought to determine if the prognostic effect of TILs differed quantitatively in patients with early-stage TNBC who were treated with anthracycline with and without taxanes, which may suggest whether the type of chemotherapy matters in their prognostic effects.

METHODS

Patient Data Sets

The study protocol of this collaborative project of selected researchers was approved by the local institutional review committee at Gustave Roussy in November 2014 and authorized by the French National Committee on Computing and Liberty.

Identified studies were prospective randomized clinical trials or large retrospective hospital series that evaluated the prognostic associations of TILs (stromal and intratumoral) in patients who were diagnosed with early-stage TNBC treated with anthracycline-based chemotherapy with or without taxane in the adjuvant setting using the same prespecified method.[6-13] All investigators and groups who had reported their series or who had initiated TIL evaluation were approached and none declined participation. Individual patient-level data were collated, checked, and pooled using individual patient data meta-analysis techniques (Fig 1).

TNBC was defined by estrogen receptor (ER), progesterone receptor (PR), and HER2 negativity, with the exception of the Gustave Roussy (GR) cohort for which PR status was not available. ER and PR status was defined for each study as per protocol definition or by central pathology review by the following percentage of immunohistochemical staining: ER and PR less than 1% for Breast International Group (BIG) 2-98, International Breast Cancer Study group (IBCSG) 22-00 (ClinicalTrials.gov identifier: NCT00022516, and Eastern Cooperative Oncology Group (ECOG) 2197 (ClinicalTrials.gov identifier: NCT00003519), and less than 10% for the others (ECOG 1199 [ClinicalTrials.gov identifier: NCT00004125], the Finland Herceptin [FinHER], European Institute of Oncology [IEO], Protocole Adjuvant dans le Cancer
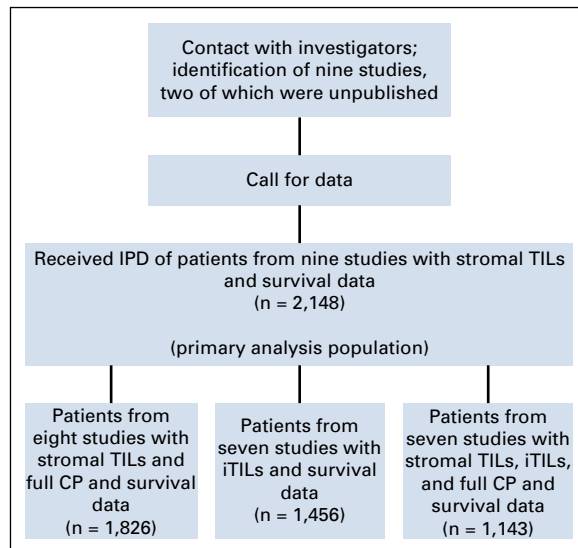

FIG 1. Study flow chart. CP, clinicopathologic; IPD, individual participant data; iTILs, intratumoral tumor-infiltrating lymphocytes; TILs, tumor-infiltrating lymphocytes.

du Sein [PACS] 01, and PACS04). ER and PR staining was performed centrally for all studies except FinHER, IEO, ECOG 2197, and ECOG 1199. For those few patients for which central ER status was missing, local ER was used. Central HER2 evaluation was performed for FinHER, IEO, IBCSG 22-00, ECOG 2197, PACS01, and PACS04 series.

Quantification of TILs

Quantification of sTILs and iTILs was performed according to International Immuno-Oncology Biomarker Working Group guidelines.[2,3] A Web site is freely available that describes the method and provides a freely available training tool for TIL assessment by pathologists (www.tilsinbreastcancer.org). TILs data in the PACS01 and PACS04 clinical trials were obtained for the purposes of this study (by R.S. and M.L.) and have not been previously published. For the pooled analysis, the sTILs variable was the primary prespecified biomarker as this has been found to be more reproducible compared with iTILs.[2,3]

Statistical Analysis

The prespecified primary end point was invasive disease-free survival (iDFS), defined as the date of first invasive recurrence, or second primary or death from any cause.[14] Patients still alive without an event of interest were censored at the date of the last visit. Distant disease-free survival (D-DFS) is defined as the date of first distant recurrence or death from any cause. Patients still alive without an event of interest were censored at the date of the last visit. Overall survival (OS) is defined as the date of death from any cause. Patients still alive were censored at the date of the last visit. We used the Kaplan-Meier method to establish survival curves and the log-rank test to compare survival curves across subgroups.

The prespecified primary analysis used sTILs as a continuous variable in the Cox proportional hazards regression model. On the basis of the BIG 2-98 data,[8] we assumed a standard deviation of 1.75 on the scale of 10% changes in sTILs in the subset of patients with TNBC. On the basis of this value, a total of 111 invasive events or deaths would provide 85% power at a 5% two-sided significance level to detect a hazard ratio (HR) of 0.85 for a 10% increase in sTILs in a Cox proportional hazards regression model on iDFS.

Univariable and multivariable risk analyses were performed using the Cox proportional hazards regression model with gamma frailty to account for the study heterogeneity. We checked the proportional hazard assumption using trend tests and graphical diagnoses on the basis of Schoenfeld residuals as well as the log-linearity assumption using fitting splines. Associations with clinicopathologic variables were performed using linear regression adjusted for study.

A Cochrane Q-like test was performed and the I^2 index was approximated for each end point to characterize the heterogeneity between each study ($I^2 = 0$ indicates no heterogeneity and larger values correspond to increasing heterogeneity). In the multivariable model, we used the likelihood ratio test to evaluate the contribution of sTILs, iTILs, and the clinicopathologic factors (continuous age; categorical tumor size: T1, T2, T3+; continuous number of positive nodes; histologic grade: 1 and 2 v 3; and chemotherapy treatment modality: anthracycline v anthracycline plus taxane). Cox models were weighted to take in account the case-control sampling in the ECOG 2197 study.[6]

The fully developed multivariable Cox proportional hazards regression model used sTILs and the clinicopathologic factors specified above using gamma frailty for study, together with the Breslow estimator of the baseline hazard. Three such models were developed for the three end points, iDFS, D-DFS, and OS. To estimate the discrimination and calibration of these multivariable prognostic models, we used a leave-one-study-out cross-validation approach. Each study was left out once and the model was rebuilt from the beginning with the other studies. This model was then applied to the omitted study to evaluate discrimination and calibration. We assessed discrimination using time-dependent area under the curve at 5 years,[15] whereas calibration was evaluated via calibration plots. During each step of the leave-one-out cross-validation, strata were defined according to deciles of the predicted survival times of the left-out trial. A good calibration corresponds to the absence of large differences between the Kaplan-Meier estimate and the mean prediction value in each of these strata.

RESULTS

Patient Characteristics

In total, nine studies comprising individual data on 2,148 patients were collected for the assessment of the prognostic value of sTILs in the primary analysis. These studies and the patient characteristics are listed in Table 1 and the Data Supplement. All but one data set[10] were prospective clinical trials. For the iDFS end point, median follow-up was 6.58 years (range, 2.71 to 9.12 years) with 736 events (Data Supplement). For the D-DFS end point, median follow-up was 6.99 years (range, 3.50 to 9.54 years) with 584 events. For the OS end point, median follow-up was 7.15 years (range, 4.18 to 9.61 years) with 533 events. Of these 2,148 patients, 1,826 (84.7%) had both sTILs and complete clinicopathologic information available for multivariable modeling (Fig 1 and Table 1).

Mean sTILs level was 23% (standard deviation, 20%; range 0% to 95%) and median was 15.00% (interquartile range, 10% to 30%). Boxplots are provided from the study and overall in the Data Supplement. Patients with lymph node–negative tumors comprised 32.9% (706 of 2,148 patients) of the cohort (mean sTIL level, 20.2%; standard deviation, 20.3%) and all had received anthracycline alone (55.8%) or anthracycline with taxane (44.2%) adjuvant chemotherapy. In total, 1,592 patients (73.8%) underwent iTILs evaluation, with 1,144 patients (53.1%) having complete clinicopathologic information available (Data Supplement). In this pooled data set, quantities of sTILs and iTILs were strongly correlated (R = 0.63; $P < .001$).

Association With Clinicopathologic Factors

sTILs quantities were significantly lower with older age (linear model, $P = .001$). There was a significant inverse relationship between larger tumor burden and the amounts of sTILs present (tumor size $P = .01$; more nodes $P = .02$; Data Supplement). Consistent with previous reports, higher grade was associated with larger TILs amounts ($P = .001$).

Associations With Prognosis

The quantity of sTILs was significantly associated with improved survival outcomes for all end points (Fig 2). For iDFS, HR for a 10% increment in sTILs was equal to 0.86 (95% CI, 0.83 to 0.90) and similar results were observed for D-DFS (0.83 [95% CI, 0.78 to 0.87]) and OS (0.83 [95% CI, 0.79 to 0.88]) with no significant heterogeneity found for each end point. There was no significant interaction between sTILs and chemotherapy treatment (ie, the magnitude of the prognostic effect was similar if patients received anthracycline alone v anthracycline-taxane regimens; P for interaction: iDFS: $P = .62$; D-DFS: $P = .63$; OS: $P = .54$).

Results of the prognostic analysis of iTILs were similar (Data Supplement), again with no significant heterogeneity between studies for each end point. Overall, a 10% increment in iTILs corresponded to HRs of 0.74 (95% CI, 0.65 to 0.85) for iDFS, 0.73 (95% CI, 0.62 to 0.84) for D-DFS, and 0.76 (95% CI, 0.65 to 0.88) for OS.

Exploratory Prognostic sTIL Cutoff

Figure 3 depicts survival curves using the exploratory sTILs level cutoff of 30%, and Figure 4 the survival curves

TABLE 1. Baseline Patient Characteristics of Include Patients With TNBC With Data on Stromal TILs Counts

Characteristic	All Patients	Patients With Complete Data on CP Factors
Size, No.	2,148	1,826
Stromal TILs, %		
Mean (SD)	23.21 (20.37)	24.32 (20.69)
Median (IQR)	15.00 (10.00-30.00)	18.00 (10.00-35.00)
Range	0.00-95.00	0.00-95.00
Intratumoral TILs, %		
Mean (SD)	5.29 (10.29)	6.11 (11.06)
Median (IQR)	1.00 (0.00-5.50)	2.00 (0.00-6.00)
Range	0.00-90.00	0.00-90.00
Missing, No. (%)	692 (32.22)	683 (37.40)
Age, years		
Mean (SD)	49.76 (10.00)	49.94 (9.83)
Median (IQR)	50.00 (42.20-57.00)	50.00 (42.92-57.00)
Range	22.00-85.00	22.00-85.00
Tumor size category, cm, No. (%)		
T1 ≤ 2	831 (38.69)	750 (41.07)
T2 > 2-5	1,176 (54.75)	983 (53.83)
T3 > 5	127 (5.91)	93 (5.09)
Missing	14 (0.65)	0 (0.00)
Positive nodes		
Mean (SD)	2.96 (4.89)	2.87 (4.90)
Median (IQR)	1.00 (0.00-3.00)	1.00 (0.00-3.00)
Range	0.00-46.00	0.00-46.00
Missing, No. (%)	1 (0.05)	0 (0.00)
Positive nodes category, No. (%)		
0	706 (32.87)	647 (35.43)
1-3	910 (42.36)	743 (40.69)
> 3	531 (24.72)	436 (23.88)
Missing	1 (0.05)	0 (0.00)
Histologic grade, No. (%)		
Grade 1	165 (7.68)	162 (8.87)
Grade 2	265 (12.34)	261 (14.29)
Grade 3	1,411 (65.69)	1,403 (76.83)
Missing	307 (14.29)	0 (0.00)
Treatment, No. (%)		
Anthracycline	1,198 (55.77)	1,181 (64.68)
Anthracycline + taxane	950 (44.23)	645 (35.32)

NOTE. Complete data on CP factors corresponds to age, tumor size, nodal status and histologic grade. Data set, [5-12] cumulative anthracycline dose: Finland Herceptin (n = 134), epirubicin 300 mg/m^2; Eastern Cooperative Oncology Group 1199 and 2197 (n = 479), doxorubicin 240 mg/m^2; Gustave Roussy (n = 107), doxorubicin 300 mg/m^2; European Institute of Oncology series (n = 292), doxorubicin 240 mg/m^2; Protocole Adjuvant dans le Cancer du Sein (PACS) 01 (n = 175), epirubicin 300 mg/m^2; PACS04 (n = 167), epirubicin 300 mg/m^2 or epirubicin 450 mg/m^2; International Breast Cancer Study Group 22-00: 85% received doxorubicin 240 mg/m^2; Breast International Group 2-98 (n = 269), doxorubicin 240 mg/m^2.

Abbreviations: CP, clinicopathologic factors; IQR, interquartile range; SD, standard deviation; TNBC, triple-negative breast cancer; TIL, tumor-infiltrating lymphocyte.
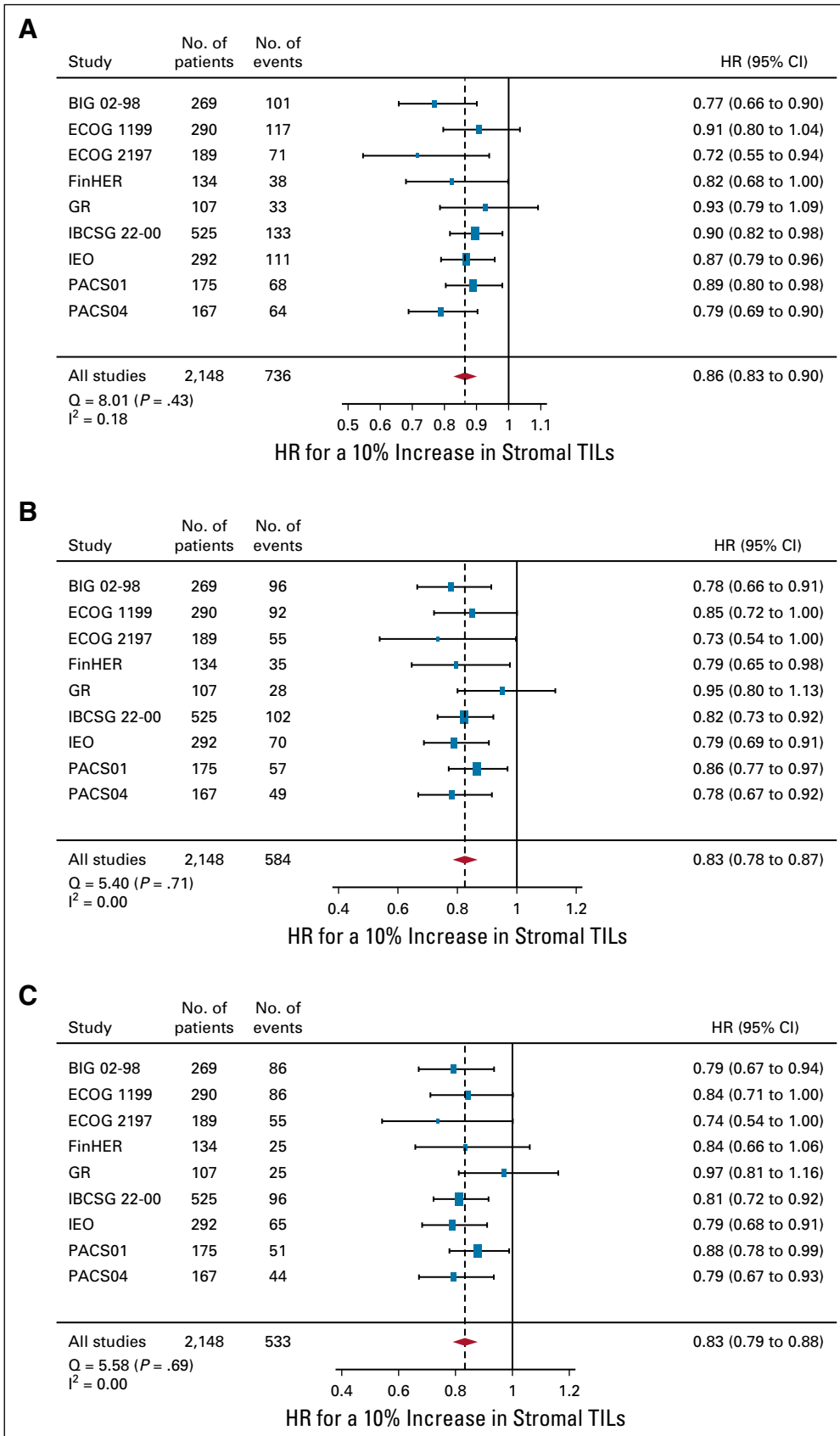
FIG 2. Forest plot of the prognostic effect of the stromal tumor-infiltrating lymphocytes (TILs) variable for each 10% increment on (A) invasive disease-free survival, (B) distant disease-free survival, and (C) overall survival. Hazard ratios (HR) are derived using Cox proportional hazards regression models and presented with 95% CIs. The size of the boxes is proportional to the inverse of the variance of the estimator in each study. Cochrane Q-like test (Q) and I^2 statistics were used to evaluate between-study heterogeneity. BIG, Breast International Group; ECOG, Eastern Cooperative Oncology Group; FinHER, Finland Herceptin; GR, Gustave Roussy; IBCSG, International Breast Cancer Study Group; IEO, European Institute of Oncology; PACS, Protocole Adjuvant dans le Cancer du Sein.

A

Study	No. of patients	No. of events	HR (95% CI)
BIG 02-98	269	101	0.77 (0.66 to 0.90)
ECOG 1199	290	117	0.91 (0.80 to 1.04)
ECOG 2197	189	71	0.72 (0.55 to 0.94)
FinHER	134	38	0.82 (0.68 to 1.00)
GR	107	33	0.93 (0.79 to 1.09)
IBCSG 22-00	525	133	0.90 (0.82 to 0.98)
IEO	292	111	0.87 (0.79 to 0.96)
PACS01	175	68	0.89 (0.80 to 0.98)
PACS04	167	64	0.79 (0.69 to 0.90)
All studies	2,148	736	0.86 (0.83 to 0.90)

Q = 8.01 (P = .43)
I^2 = 0.18

HR for a 10% Increase in Stromal TILs

B

Study	No. of patients	No. of events	HR (95% CI)
BIG 02-98	269	96	0.78 (0.66 to 0.91)
ECOG 1199	290	92	0.85 (0.72 to 1.00)
ECOG 2197	189	55	0.73 (0.54 to 1.00)
FinHER	134	35	0.79 (0.65 to 0.98)
GR	107	28	0.95 (0.80 to 1.13)
IBCSG 22-00	525	102	0.82 (0.73 to 0.92)
IEO	292	70	0.79 (0.69 to 0.91)
PACS01	175	57	0.86 (0.77 to 0.97)
PACS04	167	49	0.78 (0.67 to 0.92)
All studies	2,148	584	0.83 (0.78 to 0.87)

Q = 5.40 (P = .71)
I^2 = 0.00

HR for a 10% Increase in Stromal TILs

C

Study	No. of patients	No. of events	HR (95% CI)
BIG 02-98	269	86	0.79 (0.67 to 0.94)
ECOG 1199	290	86	0.84 (0.71 to 1.00)
ECOG 2197	189	55	0.74 (0.54 to 1.00)
FinHER	134	25	0.84 (0.66 to 1.06)
GR	107	25	0.97 (0.81 to 1.16)
IBCSG 22-00	525	96	0.81 (0.72 to 0.92)
IEO	292	65	0.79 (0.68 to 0.91)
PACS01	175	51	0.88 (0.78 to 0.99)
PACS04	167	44	0.79 (0.67 to 0.93)
All studies	2,148	533	0.83 (0.79 to 0.88)

Q = 5.58 (P = .69)
I^2 = 0.00

HR for a 10% Increase in Stromal TILs

stratified by nodal status and the cutoff. Whereas cutoffs can be difficult to choose for a variable with a linear effect on prognosis in the Cox proportional hazards regression model, this percentage was chosen as it reflected the top quartile of sTILs levels across the cohort for the three clinical end points. Estimated 3-year and 5-year survival probabilities for all three end points across subgroups and for the entire population are reported in the Data Supplement. Large absolute differences in survival outcomes are observed across all nodal categories. For all patients with node-negative TNBC with at least 30% sTILs present at diagnosis (n = 213), excellent survival can be observed with an estimated 3-year iDFS of 92% (95% CI, 89% to 98%), 3-year D-DFS of 97% (95% CI, 95% to 99%), and 3-year OS of 99% (95% CI, 97% to 100%).

When focusing on the T1-2, N0 subpopulation with at least 30% TILs—one third of the node-negative population (206 of 684 patients)—3-year estimates for iDFS, D-DFS, and OS were 93% (95% CI, 90% to 97%), 98% (95% CI, 95% to 100%), and 99% (95% CI, 98% to 100%), respectively.

Integrated Clinicopathologic Prognostic Model

Univariable and multivariable analyses confirmed the significant and independent prognostic value of both sTILs and iTILs in early-stage TNBC as a continuous variable (Table 2 and Data Supplement). Using a likelihood ratio test, we investigated the added prognostic information of TILs to standard clinical prognostic factors. sTILs added significant prognostic information beyond that provided by conventional clinicopathologic factors (likelihood ratio test χ^2, 48.9 for iDFS; χ^2, 55.8 for D-DFS; and χ^2, 48.5 for OS; all $P < .001$). In contrast, iTILs did not add additional information to the model with sTILs (Data Supplement).

An integrated prognostic model that combined standard prognostic clinicopathologic factors with continuous sTIL levels was created to provide improved estimates of survival outcomes in early-stage TNBC. Addition of the sTILs variable increased the discrimination of the model as estimated using leave-one-study-out cross-validation from a 5-year area under the curve of 0.65 (0.55; 0.71) to 0.68 (0.56; 0.76) for IDFS, from 0.67 (0.55; 0.76) to 0.69 (0.58; 0.78) for D-DFS, and from 0.68 (0.56; 0.81) to 0.69 (0.58; 0.78) for OS (Data Supplement). This prognostic model was well calibrated on all three end points in the leave-one-study-out cross-validation (Data Supplement). The model can be used by clinicians to estimate survival incorporating the sTIL biomarker and is freely accessible at the following Web site: www.tilsinbreastcancer.org.

DISCUSSION

In this study, by pooling individual patient-level data from nine large studies, we have comprehensively demonstrated that the quantity of stromal TILs at the time of diagnosis is a robust and independent prognostic factor for early-stage TNBC and adds important prognostic information to the

estimations of survival when combined with standard clinicopathologic factors. Hence, despite the complexities of the antitumor immune response, these data strongly suggest that the quantity of preexisting immune infiltrate present at diagnosis strongly correlates with outcomes in patients with early-stage TNBC treated with anthracycline-based chemotherapy. Evaluation of the stromal TILs method has been previously published and reproducibility demonstrated[3,16]; therefore, our data suggest that this new biologic biomarker is ready for clinical use and could be implemented globally for patient prognostication and clinical trial stratification. Our prognostic model is freely available online and this should thus facilitate its uptake by clinicians and pathologists world-wide, as well as for investigators who wish to externally validate the model on their TNBC data sets.[17]

In the current study, it is the quantity of baseline TILs infiltrate observed at diagnosis that is prognostic. As all patients in this study had breast surgery followed by adjuvant chemotherapy, this suggests that, despite the generation of high TILs, the primary tumor cannot be completely eradicated by the host immune system, but the effective antitumor immunity memory must still have been initiated. Immune infiltrates in primary breast cancers have been demonstrated to be predominantly T cells.[18] In support of this, the quantity of CD8+ T cells when evaluated by immunohistochemistry has also been shown to be prognostic.[16,19] Biologic mechanisms that underlie the heterogeneity observed in the quantity of the immune infiltrate and that drive the T-cell response in breast cancers are largely unknown. Somatic and germline mutations, genomic instability, intracellular antigens, and such host factors as the microbiome have all been hypothesized to contribute.[20-22] Of interest, we observed inverse relationships between TILs quantity with tumor size and nodal burden, which supports oncogenic-mediated immune evasion with larger disease burden, but increased levels with high histologic grade, which supports genomic instability as a possible trigger.[23] In general, breast cancers have low mutational burden, perhaps with the exception of *BRCA* germline mutated tumors.[24] It is currently unknown whether qualitative T-cell differences exist in high TIL TNBCs or if simply quantitative differences dictate prognosis. Additional research into the molecular basis of TIL heterogeneity is likely to be informative.

We observed strong prognostic effects of stromal TIL values on all survival end points above provided by important clinicopathologic factors. One could speculate that generating an effective antitumor immune response may result in significant survival improvements for patients with low TIL levels, though our data do not directly support this. Given that the vast majority of early-stage TNBCs contained at least 1% TILs, it would seem that immunotherapeutic agents that require a preexisting response are likely to be efficacious in this setting.[25] More research into the duration
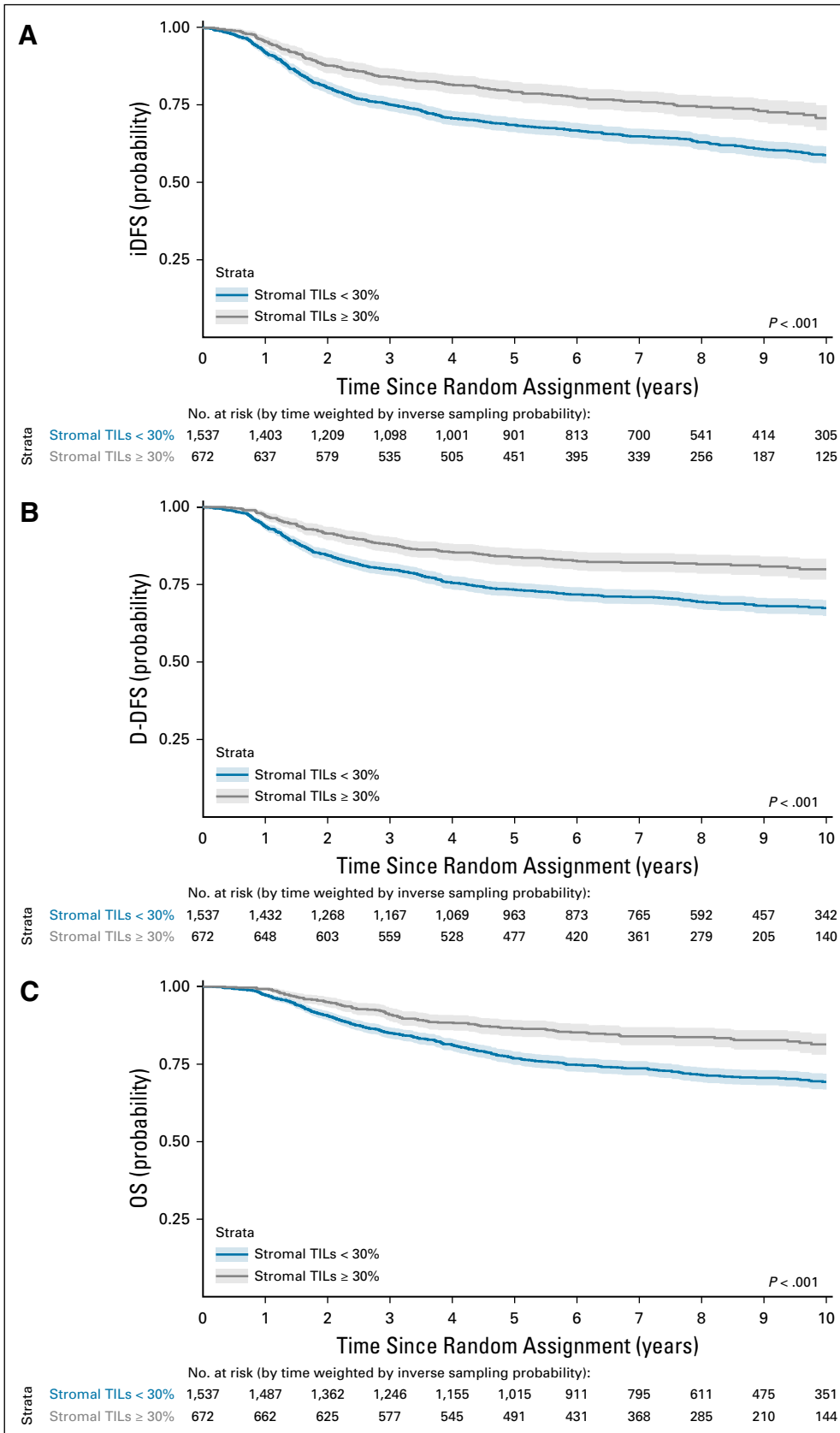
FIG 3. Kaplan-Meier curves of (A) invasive disease-free survival (iDFS), (B) distant disease-free survival (D-DFS), and (C) overall survival (OS) according to stromal tumor-infiltrating lymphocytes (TILs) dichotomized at the value of 30% in the entire population. Shaded areas correspond to pointwise 95% CIs. *P* values correspond to log-rank tests.
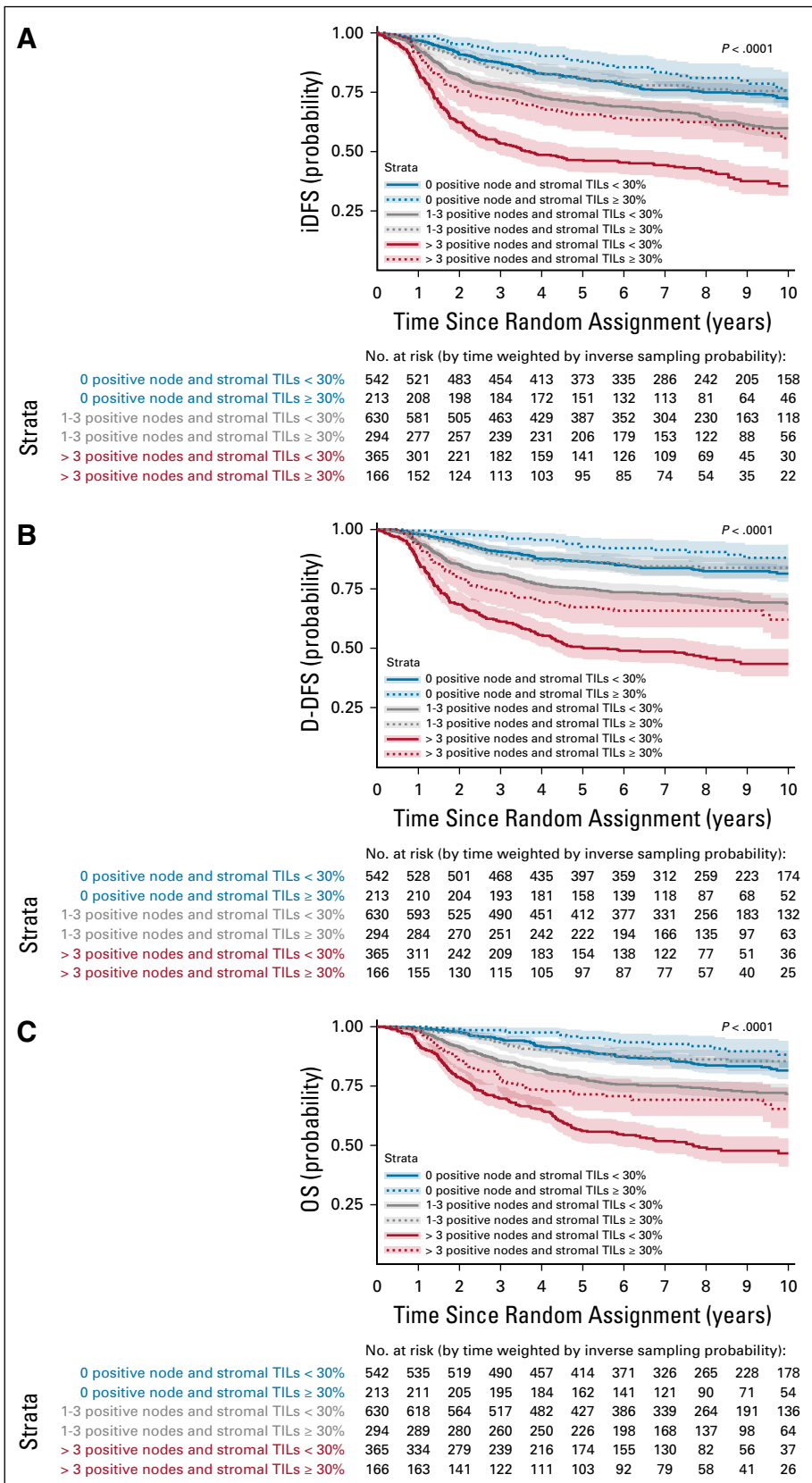
FIG 4. Kaplan-Meier curves of (A) invasive disease-free survival (iDFS), (B) distant disease-free survival (D-DFS), and (C) overall survival (OS) according to nodal status and stromal tumor-infiltrating lymphocytes (TILs) dichotomized at the value of 30%. Shaded areas correspond to pointwise 95% CIs. P values correspond to log-rank tests. iDFS estimated 3-year survival for stromal TILs ≥ 30% v < 30% for node negative disease: 92% (95% CI, 0.89 to 0.96) v 88% (95% CI, 0.85 to 0.90); estimated 5-year survival: 88% (95% CI, 0.84 to 0.93) v 81% (95% CI, 0.77 to 0.84). D-DFS estimated 3-year survival for stromal TILs ≥ 30% v < 30% for node-negative disease: 97% (95% CI, 0.95 to 0.99) v 91% (95% CI, 0.88 to 0.83); estimated 5-year survival: 93% (95% CI, 0.89 to 0.96) v 87% (95% CI, 0.84 to 0.90). OS estimated 3-year survival for stromal TILs ≥ 30% v < 30% for node-negative disease: 99% (95% CI, 0.97 to 1.00) v 95% (95% CI, 0.93 to 0.97); estimated 5-year survival: 95% (95% CI, 0.92 to 0.98) v 90% (95% CI, 0.87 to 0.92).

TABLE 2. Multivariable Survival Analyses Adjusted for Stromal TILs, Age, Tumor Size, Number of Positive Nodes, Histologic Grade, and Treatment Using a Gamma Frailty for Study

Variable (n = 1,826)	iDFS (608 events)		D-DFS (482 events)		OS (438 events)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Stromal TILs, per 10% increments	0.87 (0.83 to 0.91)	$< 10^{-6}$	0.83 (0.79 to 0.88)	$< 10^{-6}$	0.84 (0.79 to 0.89)	$< 10^{-6}$
Age, years	1.00 (1.00 to 1.01)	.394	1.00 (1.00 to 1.01)	.323	1.01 (1.00 to 1.010	.300
Tumor size category, cm						
T1 ≤ 2	1	Reference	1	Reference	1	Reference
T2 2-5	1.30 (1.10 to 1.55)	$< 10^{-2}$	1.46 (1.20 to 1.78)	$< 10^{-3}$	1.44 (1.17 to 1.77)	$< 10^{-3}$
T3 ≥ 5	1.63 (1.18 to 2.25)	$< 10^{-2}$	1.73 (1.22 to 2.46)	$< 10^{-2}$	1.61 (1.11 to 2.33)	.012
Positive nodes, N0 v N1 v N2	1.07 (1.06 to 1.08)	$< 10^{-6}$	1.08 (1.07 to 1.09)	$< 10^{-6}$	1.08 (1.07 to 1.09)	$< 10^{-6}$
Histologic grade						
Grade 1 or 2	1	Reference	1	Reference	1	Reference
Grade 3	1.05 (0.86 to 1.30)	.617	1.16 (0.91 to 1.48)	.233	1.17 (0.91 to 1.49)	.223
Treatment						
Anthracycline	1	Reference	1	Reference	1	Reference
Anthracycline + taxane	1.04 (0.87 to 1.24)	.704	0.98 (0.80 to 1.21)	.877	1.03 (0.84 to 1.27)	.771

Abbreviations: D-DFS, distant disease-free survival; HR, hazard ratio; iDFS, invasive disease-free survival; OS, overall survival; TILs, tumor-infiltrating lymphocytes.

and type of chemotherapy that can enhance or promote an immune response in combination to optimize with immune therapies, such as checkpoint blockade, is warranted.

Strengths of this study include the large number of patients pooled from multiple different studies, including large prospective clinical trials. Using an exploratory stromal TILs cutoff of 30% or more, the observed excellent survival supports the clinical validity of the TILs biomarker. This suggests that the biomarker could be incorporated into future prognostic staging systems for early-stage, node-negative TNBC, similar to the recent integration of the prognostic 21-gene assay for patients with hormone receptor–positive, HER2-negative, node-negative disease.[26] This stromal TIL score has been demonstrated to be reproducible between pathologists after adequate training.[2] A limitation is that our study does not include patients who were diagnosed in the past 10 years, which may affect survival estimates. In addition, the exact immune subset of the TILs biomarker responsible for good outcomes is not known. Whereas this study does not directly address the

question of de-escalation for the subgroup of node negative TNBC patients with high sTILs that have good prognosis when treated by anthracycline-based chemotherapy (with or without taxane), the identification of this subgroup with good prognosis can still be useful. Excluding this subgroup of high sTILs and node-negative patients may also be useful in future clinical trials of new therapeutics for early-stage TNBC through the reduction of toxicity and cost for patients with a good outcome, as well as in the required number of patients. Future prospective validation of the proposed sTIL cutoff could also investigate whether this can aid clinicians in the identification of patients with TNBC with a good prognosis who may require a less intensive type and duration of traditional cytotoxic chemotherapies.

In conclusion, we have shown that baseline stromal TIL quantities provide important and independent prognostic information in early-stage TNBC. A subgroup of patients with high-TIL, early-stage, node-negative TNBC have low rates of recurrence and death from disease at 3 years after standard anthracycline-based adjuvant chemotherapy

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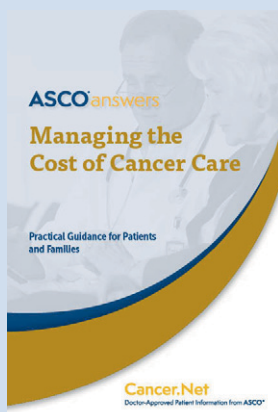
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