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ORIGINAL ARTICLE

# Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy

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**Background:** Although stromal tumor-infiltrating lymphocytes (sTILs) have been considered an important prognostic factor in early-stage triple-negative breast cancer (TNBC), there have been limited data on their prognostic value in the absence of adjuvant chemotherapy.

**Patients and methods:** A pooled analysis was carried out using four cohorts of TNBC patients not treated with chemotherapy. sTILs were evaluated in the most representative tumoral block of surgical specimens. Cox proportional hazards regression models were used for invasive disease-free survival (iDFS), distant disease-free survival (D-DFS), and overall survival (OS), fitting sTILs as a continuous variable adjusted for clinicopathologic factors.

**Results:** We analyzed individual data of 476 patients from 4 centers diagnosed between 1989 and 2015. Their median age was 64 years. The median tumor size was 1.6 cm and 83% were node-negative. The median level of sTILs was 10% (Q1−Q3, 4%−30%). Higher grade was associated with higher sTILs ($P < 10^{-3}$). During follow-up, 107 deaths, and 173 and 118 events for iDFS and D-DFS were observed, respectively. In the multivariable analysis, sTILs obtained an independent prognostic value for all end points (likelihood ratio $\chi^2 = 7.14$ for iDFS; $P < 10^{-2}$; $\chi^2 = 9.63$ for D-DFS, $P < 10^{-2}$; $\chi^2 = 5.96$ for OS, $P = 0.015$). Each 10% increment in sTILs corresponded to a hazard ratio of 0.90 [95% confidence interval (CI) 0.82 − 0.97] for iDFS, 0.86 (95% CI 0.77 − 0.95) for D-DFS, and 0.88 (95% CI 0.79 − 0.98) for OS, respectively. In patients with pathological stage I tumors with sTILs ≥30% ($n = 74$), 5-year iDFS was 91% (95% CI 84% to 96%), D-DFS was 97% (95% CI 93% to 100%), and OS was 98% (95% CI 95% to 100%).

**Conclusion:** sTILs add important prognostic information in systemically untreated early-stage TNBC patients. Notably, sTILs can identify a subset of stage I TNBC patients with an excellent prognosis without adjuvant chemotherapy.

**Key words:** triple-negative breast cancer, tumor-infiltrating lymphocytes, prognosis, adjuvant chemotherapy

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## Introduction

Triple-negative breast cancer (TNBC) is a heterogeneous group of breast tumors defined by the lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, accounting for 10%−15% of breast cancer (BC) [1, 2]. Compared with other BC subtypes, TNBC patients have a more aggressive disease phenotype with poorer survival outcomes [3]. With no clinically useful biomarkers or personalized targeted therapies yet available for TNBC, cytotoxic chemotherapy remains the standard systemic treatment of early TNBC, although the optimal regimen has not yet been established [4]. While TNBC as an entity presents a poor prognosis with a relatively high risk of relapse in the first 5 years [5], results from observational studies have shown that a subset of TNBC patients with very small (<1 cm) and node-negative disease have a good prognosis, with a 5-year risk of distant relapse of <10% [6]. Thus, omission of chemotherapy may be considered in these low-risk patients, if they can be correctly identified.

Tumor-infiltrating lymphocytes (TILs), a surrogate marker of adaptive immune response, have shown their association with improved prognosis in early-stage BC [7, 8]. In TNBC patients, a higher quantity of TILs has been identified as a biomarker of increased pathological response after neoadjuvant chemotherapy and prognostic factor in patients treated with adjuvant chemotherapy [7, 9]. In a latest pooled analysis of patients treated with chemotherapy, node-negative TNBC patients with at least 30% sTILs had excellent survival, raising the possibility of using sTILs to identify a subgroup of TNBC patients with good prognosis who may need less or no systemic chemotherapy [9]. However, data regarding the prognostic effect of TILs in the absence of chemotherapy has been limited. A recent retrospective study of a small number of TNBC patients who were not treated with adjuvant chemotherapy reported that both stromal and intratumoral TILs were associated with invasive disease-free survival (iDFS) and stromal TILs (sTILs) with overall survival (OS), suggesting the prognostic role of TILs regardless of adjuvant chemotherapy [10].

The objective of the study was to evaluate intrinsic prognostic value of sTILs in patients with early stage TNBC who did not receive adjuvant chemotherapy using a pooled analysis of individual patient data. We also evaluated whether sTILs could identify a subgroup of TNBC patients with excellent absolute prognosis, for whom adjuvant chemotherapy might be withheld.

## Patients and methods

### Study design

The study was initiated by Gustave Roussy (GR), who contacted three other institutes in order to reach the necessary patient sample size (see Statistical analysis section). We collected individual patient data from a total of four centers (Table 1), which consisted of a prospective randomized clinical trial (GR, Villejuif, France) [11] and three retrospective single-center series [Institut Curie, Paris, France; Istituto Europeo di Oncologia (IEO), Milan, Italy; Asan Medical Center, Seoul, South Korea]. For the analysis, we included patients with TNBC who did not receive adjuvant chemotherapy, and excluded those with missing TILs values or follow-up data. TNBC was defined by ER, PR, and HER2 negativity, except for the GR study for which PR status was not available. ER, PR, and HER2 status was evaluated in each hospital according to local protocol (see supplementary Appendix S1, available at *Annals of Oncology* online). Quantification of TILs was carried out according to International Immuno-Oncology Biomarker Working Group guidelines [12, 13] (see http://www.tilsinbreastcancer.org).

### Statistical analysis

The primary end point was iDFS, defined as the time from surgery to the occurrence of first invasive (local or regional) or distant event, contralateral, or second primary tumors or death from any cause. Patients still alive without an event of interest were censored at the date of the last visit. Distant disease-free survival (D-DFS) was defined as the time to the first distant recurrence, second primary tumors, or death from any cause. Patients still alive without an event of interest were censored at the date of the last visit. OS was defined as the time from surgery to the date of death from any cause.

Associations of these end points with clinicopathologic variables were carried out using Spearman's correlation for continuous variables and Kendall's $\tau$ for categorical variables. We used pairwise complete observations for the handling of missing data. Correlation coefficient values and confidence intervals (CIs) were obtained with bootstrap method.

Based on the pooled analysis of TILs in TNBC [9], we assumed a standard deviation (SD) of 2 on the scale of 10% changes in sTILs. A total of 85 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. The Cox regression models were used to evaluate the added independent prognostic value of sTILs to standard clinicopathologic factors [continuous age; continuous tumor size; continuous number of lymph nodes (LNs); tumor grade: well differentiated, moderately differentiated and poorly differentiated; and radiotherapy treatment: yes/no] through the use of likelihood ratio tests. The prognostic value of sTILs was tested by comparing likelihood values between model 1 (sTILs, univariable) and the null model, and the additional prognostic value of TILs was tested by comparing likelihood values between models 2 (clinicopathologic factors) and 3 (clinicopathologic factors plus sTILs). For each model, adjusted HRs, associated 95% CI, and *P*-value were calculated for each variable. We evaluated the proportional hazard assumption using trend tests and graphical diagnoses based on Schoenfeld residuals as well as the log-linearity assumption using fitting linear tail-restricted cubic splines. The Cox regression models were stratified by study. To estimate the discrimination and calibration of the multivariable prognostic models, we used a leave-one-study-out cross-validation approach [9].

In our previous TNBC report analyzing patients treated by anthracycline-based chemotherapy [9], we defined a cut-off of 30% based on the top quartile of the large dataset of 2148 TNBC patients. A reanalysis of three ring studies of TILs shows good concordance among pathologists when using this cut-off (unpublished manuscript submitted by the TILs Working Group). We used the Kaplan−Meier method to establish survival curves. Confidence intervals for survival probabilities were calculated using a percentile bootstrap method (1500 resamples). All analysis was carried out using R (https://www.R-project.org/).

## Results

### Patient characteristics

In total, individual data of 518 patients were collected from four centers. We excluded 42 patients with sTILs values not assessed ($n = 16$), without follow-up data ($n = 20$), and who received

**Table 1. Patient's characteristics**

| | Gustave Roussy *n* = 95 | Institut Curie *n* = 150 | Italy *n* = 159 | Korea *n* = 72 | Overall *n* = 476 |
|---|---|---|---|---|---|
| Period of inclusion | | | | | |
| Date of surgery | | | | | |
| Min—Max | 1989 — 1995 | 2005 — 2013 | 1995 — 2015 | 1999 — 2012 | 1989 — 2015 |
| Demographics | | | | | |
| Age | | | | | |
| Mean | 50.5 | 72.4 | 66.0 | 57.3 | 63.6 |
| SD | 10.0 | 12.9 | 14.2 | 14.7 | 15.4 |
| Median | 50.5 | 76.0 | 67.0 | 57.0 | 65.0 |
| Q1—Q3 | 43.4 — 57.8 | 62.3 — 82.0 | 55.0 — 76.5 | 46.0 — 70.0 | 52.0 — 77.0 |
| Min—Max | 29.1 — 69.5 | 37.0 — 94.0 | 27.0 — 96.0 | 24.0 — 81.0 | 24.0 — 96.0 |
| *n* | 95 | 150 | 159 | 72 | 476 |
| Missing | 0 | 0 | 0 | 0 | 0 |
| Biomarker information | | | | | |
| Stromal TILs | | | | | |
| Mean | 18.6 | 27.2 | 13.0 | 27.4 | 20.7 |
| SD | 18.5 | 24.1 | 18.9 | 31.6 | 23.7 |
| Median | 10.0 | 20.0 | 4.0 | 10.0 | 10.0 |
| Q1—Q3 | 5.0–25.0 | 6.3–40.0 | 2.0–15.0 | 1.0–50.0 | 4.0–30.0 |
| Min—Max | 0.0–80.0 | 0.0–90.0 | 0.0–80.0 | 1.0–90.0 | 0.0–90.0 |
| *n* | 95 | 150 | 159 | 72 | 476 |
| Missing | 0 | 0 | 0 | 0 | 0 |
| Tumor information | | | | | |
| Tumor size (cm) | | | | | |
| Mean | 2.1 | 2.0 | 1.6 | 1.8 | 1.9 |
| SD | 0.9 | 1.5 | 1.6 | 1.6 | 1.5 |
| Median | 2.0 | 1.7 | 1.2 | 1.5 | 1.6 |
| Q1—Q3 | 1.6–2.5 | 0.8–3.0 | 0.3–2.3 | 0.6–2.1 | 0.7–2.5 |
| Min—Max | 0.0–5.0 | 0.3–8.0 | 0.0–10.7 | 0.2–10.0 | 0.0–10.7 |
| *n* | 94 | 150 | 152 | 72 | 468 |
| Missing | 1 | 0 | 7 | 0 | 8 |
| Number of lymph nodes | | | | | |
| Mean | 0.9 | 0.6 | 1.0 | 0.3 | 0.7 |
| SD | 2.7 | 2.2 | 4.1 | 1.3 | 2.9 |
| Median | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Q1—Q3 | 0.0–0.5 | 0.0–0.0 | 0.0–0.0 | 0.0–0.0 | 0.0–0.0 |
| Min—Max | 0.0–21.0 | 0.0–20.0 | 0.0–38.0 | 0.0–9.0 | 0.0–38.0 |
| *n* | 95 | 149 | 128 | 72 | 444 |
| Missing | 0 | 1 | 31 | 0 | 32 |
| Lymph node status | | | | | |
| 0, *n* (%) | 71 ( 74.7 ) | 124 ( 83.2 ) | 109 ( 85.2 ) | 63 ( 87.5 ) | 367 ( 82.7 ) |
| 1 (1–3 nodes), *n* (%) | 20 ( 21.1 ) | 16 ( 10.7 ) | 11 ( 8.6 ) | 7 ( 9.7 ) | 54 ( 12.2 ) |
| 2 (>3 nodes), *n* (%) | 4 ( 4.2 ) | 9 ( 6 ) | 8 ( 6.2 ) | 2 ( 2.8 ) | 23 ( 5.2 ) |
| Missing | 0 | 1 | 31 | 0 | 32 |
| Histological grade | | | | | |
| 1, *n* (%) | 2 ( 2.1 ) | 29 ( 20.6 ) | 22 ( 14.4 ) | 2 ( 2.8 ) | 55 ( 12 ) |
| 2, *n* (%) | 44 ( 46.8 ) | 51 ( 36.2 ) | 54 ( 35.3 ) | 26 ( 36.6 ) | 175 ( 38.1 ) |
| 3, *n* (%) | 48 ( 51.1 ) | 61 ( 43.3 ) | 77 ( 50.3 ) | 43 ( 60.6 ) | 229 ( 49.9 ) |
| Missing | 1 | 9 | 6 | 1 | 17 |
| Treatment | | | | | |
| Radiotherapy | | | | | |
| 0, *n* (%) | 15 ( 15.8 ) | 41 ( 27.3 ) | 48 ( 30.2 ) | 42 ( 58.3 ) | 146 ( 30.7 ) |
| 1, *n* (%) | 80 ( 84.2 ) | 109 ( 72.7 ) | 111 ( 69.8 ) | 30 ( 41.7 ) | 330 ( 69.3 ) |
| Missing | 0 | 0 | 0 | 0 | 0 |

*Continued*

**Table 1.** *Continued*

| | Gustave Roussy *n* = 95 | Institut Curie *n* = 150 | Italy *n* = 159 | Korea *n* = 72 | Overall *n* = 476 |
|---|---|---|---|---|---|
| Number of events | | | | | |
| iDFS | | | | | |
| *n* (%) | 56 ( 58.9 ) | 33 ( 22 ) | 61 ( 38.4 ) | 23 ( 31.9 ) | 173 ( 36.3 ) |
| DDFS | | | | | |
| *n* (%) | 39 ( 41.1 ) | 27 ( 18 ) | 38 ( 23.9 ) | 14 ( 19.4 ) | 118 ( 24.8 ) |
| OS | | | | | |
| *n* (%) | 36 ( 37.9 ) | 23 ( 15.3 ) | 36 ( 22.6 ) | 12 ( 16.7 ) | 107 ( 22.5 ) |
| Follow-up (OS) | | | | | |
| Follow-up (years) | | | | | |
| Median[a] | 22.1 | 5.6 | 9.9 | 6.3 | 8.0 |
| Confidence interval 95%[a] | 21.6–23.2 | 4.5–6.3 | 8.9–10.9 | 5.8–7.4 | 7.4–9.0 |
| Min−Max | 0.9–25.8 | 0.1–13.0 | 0.0–21.7 | 1.8–17.6 | 0.0–25.8 |

[a]Calculated with reverse Kaplan−Meier method.

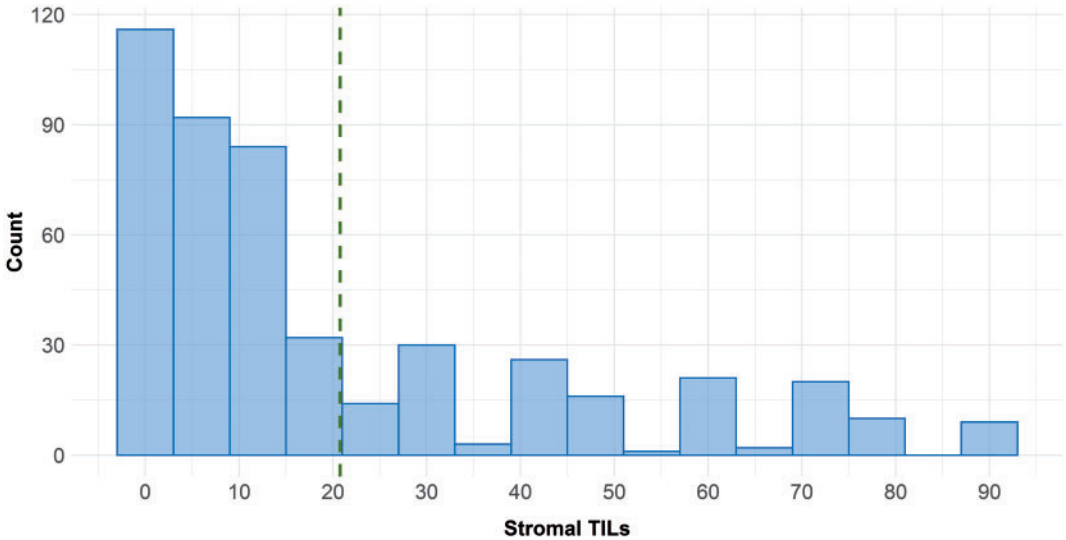


**Figure 1.** Distribution of the stromal tumor-infiltrating lymphocytes (sTILs) in the entire population. The mean is shown by the dashed line.

doxiflirudine ($n = 6$). Overall, 476 patients were included in the analysis from the 4 centers: GR ($n = 95$), Curie ($n = 150$), IEO ($n = 159$), and Asan ($n = 72$). Patient characteristics are shown in Table 1. Average age was 64 years (range 24−96 years), and 83% of patients were node-negative (368 of 444 patients with available information). All patients had undergone surgery (27% mastectomy and 73% lumpectomy) and 69% (146 of 476 patients) were treated with radiotherapy. Mean sTILs level was 21% (SD, 24%; range 0%−90%) and median was 10% (interquartile range 4%−30%) (Figure 1).

A total of 173 iDFS and 118 D-DFS events were observed. Median follow-up duration was 8 years (range 8−26 years) for OS with 107 documented deaths. sTILs quantities were significantly associated with higher grade ($P < 10^{-3}$), but not with age ($P = 0.50$), tumor size ($P = 0.48$), or number of LN ($P = 0.38$).

## Association with prognosis

The quantity of sTILs was significantly associated with improved survival outcomes for all three end points (Figure 2). Each 10% increment in sTILs corresponded to an HR of 0.93 for iDFS (95% CI 0.87−1.00), 0.89 for D-DFS (95% CI 0.81−0.98), and 0.91 for OS (0.82−1.00) (as provided in Table 2, likelihood ratio test $\chi^2_1 = 5.91$ for iDFS, $P = 0.051$; $\chi^2_1 = 5.91$ for D-DFS, $P = 0.015$; and $\chi^2_1 = 3.90$ for OS, $P = 0.048$).

The multivariable analyses confirmed an independent prognostic value of sTILs as a continuous variable in TNBC not treated with adjuvant chemotherapy (Table 2). Each 10% increment in sTILs corresponded to an HR of 0.90 (95% CI 0.83−0.98) for iDFS, 0.86 (95% CI 0.77−0.95) for D-DFS, and 0.88 (95% CI 0.79−0.98) for OS. sTILs added significant prognostic information beyond that provided by standard

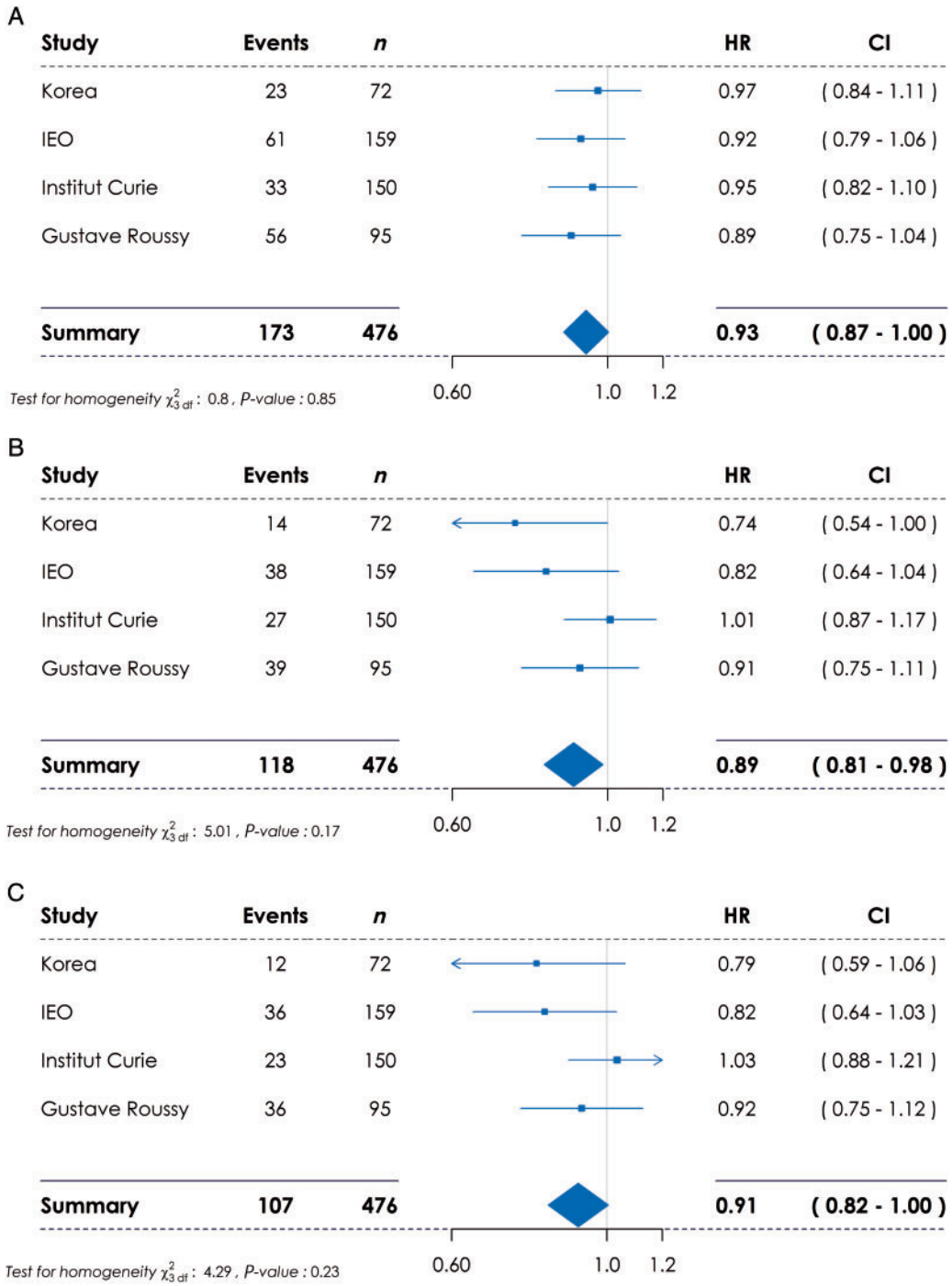


**Figure 2.** Forest plot of the prognostic effect of the stromal tumor-infiltrating lymphocytes (sTILs) variable for each 10% increment in (A) invasive disease-free survival, (B) distant disease-free survival, and (C) overall survival. Hazard ratios (HR) are derived using Cox proportional hazards regression models and presented with 95% CIs.

clinicopathologic factors (likelihood ratio test $\chi^2_1 = 3.35$ for iDFS, $P = 0.012$; $\chi^2_1 = 9.63$ for D-DFS, $P < 10^{-2}$; and $\chi^2_1 = 5.96$ for OS, $P = 0.015$). The prognostic models were well calibrated on all three end points in the leave-one-study-out cross-validation (supplementary Figure S1, available at *Annals of Oncology* online), and all continuous variables included in the model were tested for their linear effect (supplementary Figure S2, available at *Annals of Oncology* online).

## Survival probabilities by sTILs levels and tumor stage

Figure 3 shows survival curves using the sTILs level cut-off of 30%, which was defined in our previous pooled analysis of TILs in TNBC patients treated by anthracycline-based chemotherapy [9]. About one-third of the patients (138 of 476, 29%) had at least 30% sTILs. When focusing on the pathologically stage I subpopulation by AJCC 8th edition, excellent

**Table 2. Survival analysis of TILs in a Cox model stratified by study, adjusted or not on clinicopathological factors**

**Stromal TILs—iDFS**

| | **Model 1** ***n*** **= 476** ***e*** **= 173** | **Model 2** ***n*** **= 421** ***e*** **= 151** | **Model 3** ***n*** **= 421** ***e*** **= 151** |
|---|---|---|---|
| Age at randomization | – | 1.01 [0.99; 1.02] $P = 0.24$ | 1.01 [0.99; 1.02] $P = 0.31$ |
| Tumor size | – | 1.26 [1.13; 1.40] $P < 10^{-4}$ | 1.26 [1.13; 1.41] $P < 10^{-4}$ |
| Tumor grade I | – | 1 | 1 |
| Tumor grade II | – | 1.60 [0.67; 3.85] $P = 0.29$ | 1.69 [0.70; 4.08] $P = 0.24$ |
| Tumor grade III | – | 2.24 [0.94; 5.35] $P = 0.07$ | 2.74 [1.13; 6.63] $P = 0.03$ |
| Lymph node | – | 1.06 [1.02; 1.10] $P < 10^{-3}$ | 1.06 [1.03; 1.10] $P < 10^{-3}$ |
| Radiotherapy: no | – | 1 | 1 |
| Radiotherapy: yes | 1 | 0.55 [0.38; 0.80] $P < 10^{-2}$ | 0.56 [0.39; 0.83] $P < 10^{-2}$ |
| Stromal TILs(10% increment) | 0.93 [0.87; 1.00] $P = 0.06$ | – | 0.90 [0.83; 0.98] $P = 0.02$ |
| Likelihood ratio | $P$-value = 0.051 ($\chi^2_1 = 3.81$) | $P$-value = 0.012 ($\chi^2_1 = 6.35$) | |

**Stromal TILs—DDFS**

| | **Model 1** ***n*** **= 476** ***e*** **= 118** | **Model 2** ***n*** **= 421** ***e*** **= 103** | **Model 3** ***n*** **= 421** ***e*** **= 103** |
|---|---|---|---|
| Age at randomization | – | 1.03 [1.01; 1.05] $P < 10^{-2}$ | 1.03 [1.01; 1.05] $P < 10^{-2}$ |
| Tumor size | – | 1.24 [1.11; 1.40] $P < 10^{-3}$ | 1.25 [1.12; 1.40] $P < 10^{-3}$ |
| Tumor grade I | – | 1 | 1 |
| Tumor grade II | – | 2.94 [0.68; 12.60] $P = 0.15$ | 3.17 [0.74; 13.60] $P = 0.12$ |
| Tumor grade III | – | 4.37 [1.02; 18.65] $P = 0.05$ | 5.77 [1.34; 24.83] $P = 0.02$ |
| Lymph node | – | 1.06 [1.03; 1.10] $P < 10^{-3}$ | 1.07 [1.03; 1.10] $P < 10^{-3}$ |
| Radiotherapy: no | – | 1 | 1 |
| Radiotherapy: yes | 1 | 0.52 [0.33; 0.82] $P < 10^{-2}$ | 0.55 [0.35; 0.88] $P = 0.01$ |
| Stromal TILs (10% increment) | 0.89 [0.81; 0.98] $P = 0.02$ | – | 0.86 [0.77; 0.95] $P < 10^{-2}$ |
| Likelihood ratio | $P$-value = 0.015 ($\chi^2_1 = 5.91$) | $P$-value $< 10^{-2}$ ($\chi^2_1 = 9.63$) | |

**Stromal TILs—OS**

| | **Model 1** ***n*** **= 476** ***e*** **= 107** | **Model 2** ***n*** **= 421** ***e*** **= 95** | **Model 3** ***n*** **= 421** ***e*** **= 95** |
|---|---|---|---|
| Age at randomization | – | 1.03 [1.01; 1.05] $P < 10^{-2}$ | 1.03 [1.01; 1.05] $P < 10^{-2}$ |
| Tumor size | – | 1.26 [1.12; 1.43] $P < 10^{-2}$ | 1.27 [1.12; 1.43] $P < 10^{-3}$ |
| Tumor grade I | – | 1 | 1 |
| Tumor grade II | – | 2.59 [0.60; 11.20] $P = 0.20$ | 2.74 [0.63; 11.87] $P = 0.18$ |
| Tumor grade III | – | 4.13 [0.96; 17.69] $P = 0.06$ | 5.14 [1.19; 22.22] $P = 0.03$ |
| Lymph node | – | 1.10 [1.06; 1.15] $P < 10^{-2}$ | 1.11 [1.06; 1.15] $P < 10^{-5}$ |
| Radiotherapy: no | – | 1 | 1 |
| Radiotherapy: yes | 1 | 0.44 [0.27; 0.71] $P < 10^{-3}$ | 0.46 [0.28; 0.75] $P < 10^{-2}$ |
| Stromal TILs (10% increment) | 0.91 [0.82; 1.00] $P = 0.06$ | – | 0.88 [0.79; 0.98] $P = 0.02$ |
| Likelihood ratio | $P$-value = 0.048 ($\chi^2_1 = 3.90$) | $P$-value = 0.015 ($\chi^2_1 = 5.96$) | |

Model 1: sTILs, univariable; Model 2: clinicopathologic factors; Model 3: clinicopathologic factors plus sTILs.
*n*, number of patients; *e*, number of events.

survival was observed in patients with sTILs ≥30% ($n = 74$), with an estimated 5-year iDFS of 91% (95% bootstrap CI 84% to 96%), D-DFS of 97% (95% CI 93% to 100%), and OS of 98% (95% CI 95% to 100%) (Figure 4). Survival curves for the T2 subpopulation and by nodal categories are shown in supplementary Figures S3–S5, available at *Annals of Oncology* online. In node-negative patients with sTILs ≥30% ($n = 104$), 5-year iDFS was 85% (95% bootstrap CI 79% to 91%), D-DFS was 92% (95% CI 87% to 96%), and OS was 92% (95% CI 88% to 97%).

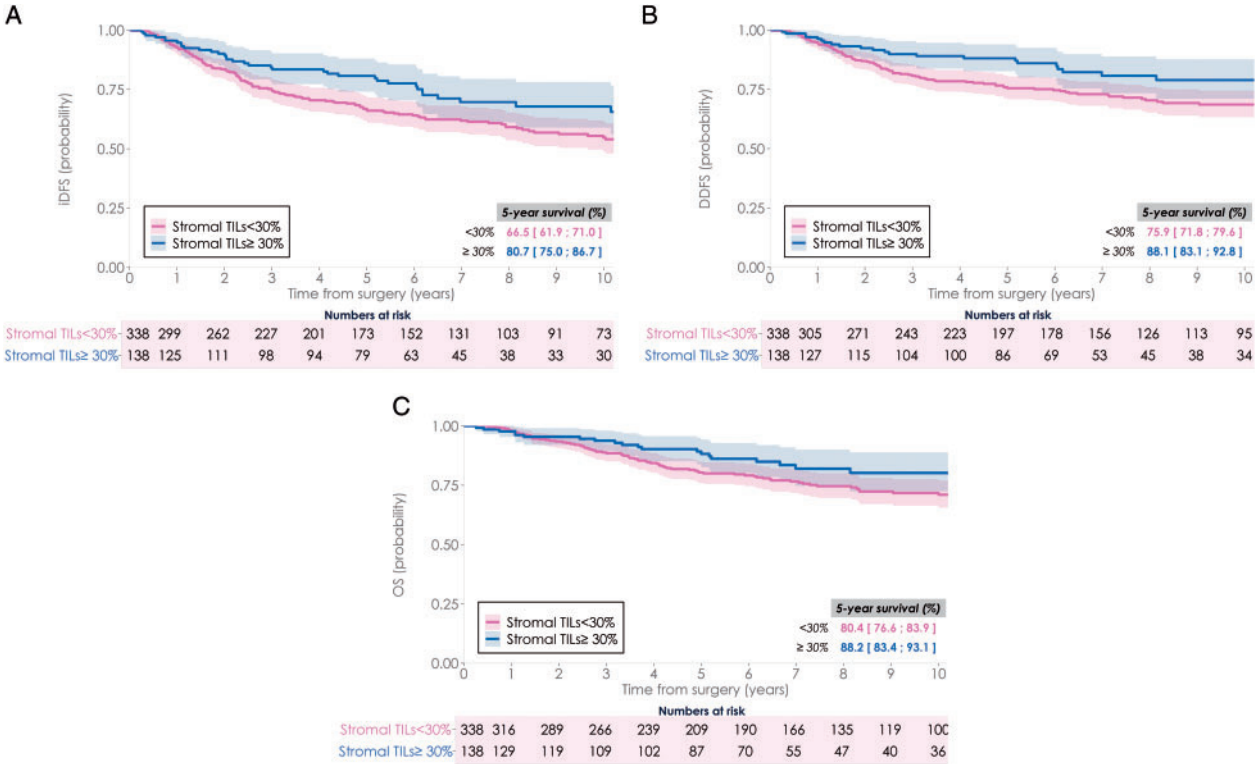


**Figure 3.** Kaplan—Meier curves of (A) invasive disease-free survival (iDFS), (B) distant disease-free survival (D-DFS), and (C) overall survival (OS) according to stromal tumor-infiltrating lymphocytes (sTILs) dichotomized at the value of 30% in the entire population. Shaded areas correspond to 95% confidence bounds. The point estimates of 5-year survival probabilities are provided with bootstrap confidence intervals.

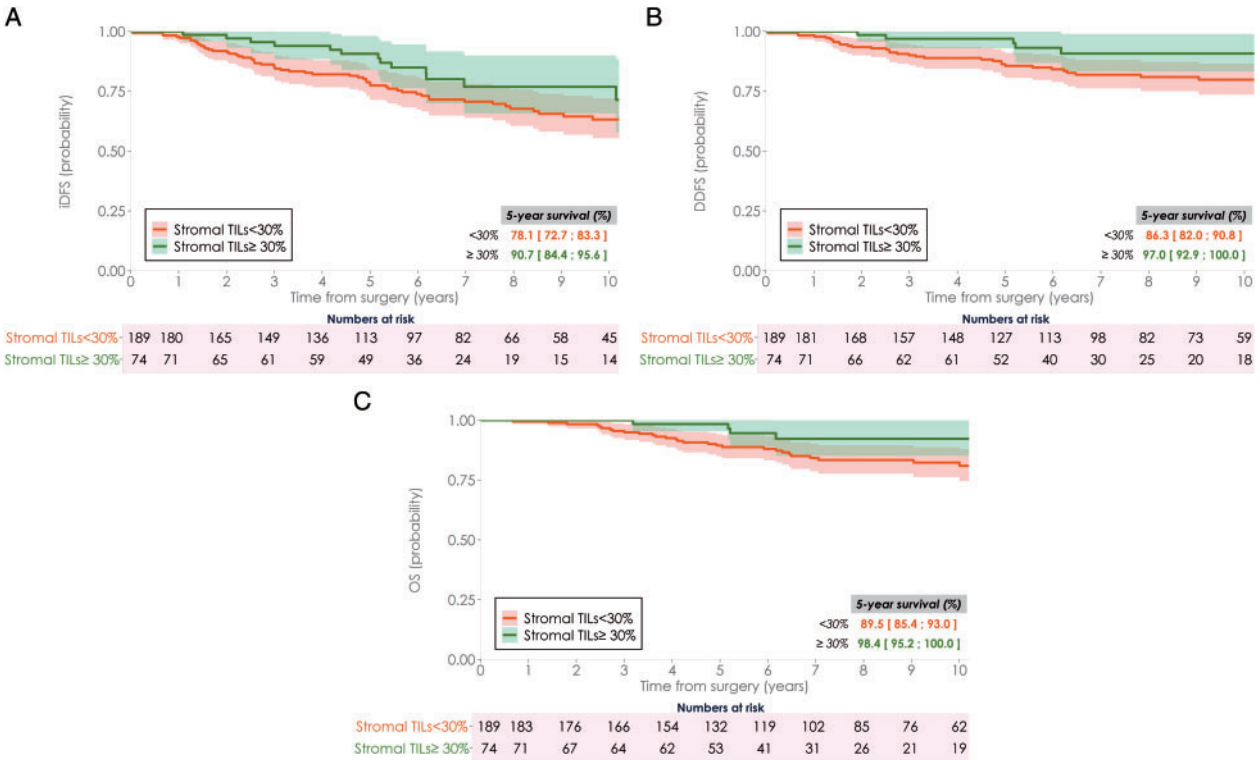


**Figure 4.** Kaplan—Meier curves of (A) invasive disease-free survival (iDFS), (B) distant disease-free survival (D-DFS), and (C) overall survival (OS) in stage I subpopulation according to stromal tumor-infiltrating lymphocytes (sTILs) dichotomized at the value of 30%.

## Discussion

In this pooled analysis of individual patient data from four series, we have shown that the quantity of sTILs at diagnosis is a significant prognostic factor in early-stage TNBC treated only with surgery with or without radiotherapy, and adds important prognostic information to current standard clinicopathologic factors. The prognostic impact of continuous sTILs on survival outcomes in this study is consistent with previous findings in early-stage TNBC patients treated with anthracycline-based chemotherapy [9, 14, 15]. In a recent retrospective study that included 182 systemically untreated TNBC patients [10], the risk of a iDFS event in patients with >50% stromal or intratumoral TILs was reduced as a half compared with those with <50% TILs (HR =0.47; 95% CI 0.24–0.90).

Treatment de-escalation in TNBC patients has been challenging due to the limited treatment options beyond cytotoxic chemotherapy and the lack of prognostic biomarkers apart from pathologic stage [6]. Our results showed that stage I TNBC patients with ≥30% sTILs had excellent survival outcomes in the absence of adjuvant chemotherapy and suggest that a subset of TNBC patients could be spared adjuvant chemotherapy since the expected survival benefit on an absolute risk scale may not outweigh the associated morbidity. Sparing adjuvant chemotherapy would be particularly useful in patients with high toxicity risk, such as elderly or comorbid patients. sTILs may therefore aid clinicians in the identification of stage I TNBC patients with excellent prognosis in whom omission of adjuvant chemotherapy may be considered. Notably these survival outcomes are similar to that reported in our previous pooled analysis in node-negative patients receiving third generation chemotherapy regimens [9]. Future prospective studies could further evaluate and validate the clinical outcome of this group having high-TIL stage I TNBC, and whether the burden of toxicity and costs can be reduced without sacrificing survival outcomes. Studies on the inter-pathologist reliability of sTILs suggest that the level of 30% is highly reproducible amongst pathologists (submitted manuscript by the TILS Working group: www.tilsinbreastcancer.org).

Our study is limited by its retrospective nature; however, we were able to include a large number of systemically untreated TNBC patients by pooling four different series, one from a prospective clinical trial and three from single hospital series. Given that neoadjuvant and/or adjuvant chemotherapy are routinely recommended in TNBC patients, our study provides a unique data source to evaluate the natural outcome of systemically untreated early-stage TNBC. Another limitation may be the potential selection bias presented by the patient population who were excluded from the systemic treatment of any reason, except for the GR study, which is comprised of patients enrolled in a randomized clinical trial of chemotherapy.

In conclusion, we have shown that baseline sTILs provide significant and independent prognostic information in patients with early-stage TNBC in the absence of adjuvant chemotherapy. We conclude that this data may confer the first clinical utility of sTILs to identify a subgroup of stage I TNBC patients who will have an excellent prognosis without chemotherapy.

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