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Role of Tumor-Infiltrating Lymphocytes in Early-Stage Triple-Negative Breast Cancer Treated with KEYNOTE-522 in Real-World Setting

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Abstract

Tumor-infiltrating lymphocytes (TILs) are established prognostic and predictive biomarkers in early-stage triple-negative breast cancer (eTNBC), yet their role in patients treated with the KEYNOTE-522 (KN522) regimen remains underexplored. The Neo-Real/GBECAM-0123 study is a real-world cohort of patients with early-stage TNBC treated with pembrolizumab plus neoadjuvant chemotherapy across Brazil and Argentina since 2020; we evaluated baseline stromal TILs (<30%, 30–49%, or ≥50%) as predictors of pathologic complete response (pCR, primary endpoint) and event-free survival (EFS, secondary endpoint). Among 248 patients, TILs were <30% in 72.6%, 30–49% in 12.9%, and ≥50% in 14.5%. pCR rose with increasing TILs: 59%, 65.6%, and 91.4% (P=0.001). In multivariable analysis, TILs ≥50% (OR 6.96, 95% CI 1.89–25.65, P=0.004) and Ki-67 ≥50% (OR 4.89, 95% CI 2.31–10.33, P<0.001) independently predicted pCR; virtually all patients with both TILs ≥50% and Ki-67 ≥50% achieved pCR (n=26/27, 96.3%). Among patients with pCR, 2-year EFS was uniformly high across TIL groups (97.0%, 95.2%, 93.2%; P=0.828); among those with residual disease, EFS trended higher with increasing TILs (73.3%, 90.0%, 100%; P=0.350), non-significant. Baseline TILs, especially with Ki-67, emerged as a strong predictor of pCR in this real-world eTNBC cohort treated with KN522, supporting their systematic assessment in future studies.

Introduction

Triple-negative breast cancer (TNBC) carries a significantly elevated risk of early recurrence and distant metastasis compared to other breast cancer subtypes, posing substantial clinical challenges (1). Advances in systemic therapy over the past decade have meaningfully improved outcomes for patients with early-stage TNBC. The integration of immunotherapy with pembrolizumab represented a major paradigm shift in this setting. In the phase 3 KEYNOTE-522 trial, the addition of pembrolizumab to a four-drug chemotherapy backbone comprising paclitaxel, carboplatin, and anthracycline-cyclophosphamide resulted in significant improvements in pCR rates (64.8% vs. 51.2%), event-free survival (EFS), and overall survival (OS), establishing this regimen as the current standard of care for stage II–III TNBC (2,3).

Alongside therapeutic advances, our understanding of the biological heterogeneity of TNBC has grown considerably, leading to the identification of biomarkers capable of distinguishing patients with more favorable outcomes. Among these, tumor-infiltrating lymphocytes (TILs) have emerged as the most promising biomarker in early-stage TNBC. TILs reflect an active endogenous antitumor immune response and have been consistently shown to carry prognostic significance across multiple cohorts. Notably, large retrospective studies have demonstrated that patients with high baseline TILs can achieve excellent long-term outcomes even in the absence of systemic chemotherapy (4–6). In the Netherlands-based PARADIGM cohort, young women (under 40 years) with node-negative TNBC who had not received neoadjuvant or adjuvant chemotherapy and had stromal TILs ≥75% showed a 15-year cumulative incidence of distant metastasis or death of only 2.1%, compared to 38.4% in those with TILs <30% (4). Furthermore, a pooled retrospective analysis including 1,966 chemotherapy-naive patients with early-stage TNBC across

13 international centers demonstrated that a TIL level of ≥50% at diagnosis was associated with a 5-year OS of 95%, compared to 82% in those with TIL levels below 30% (5).

Beyond their prognostic role, TILs have also been consistently identified as a predictor of pathologic complete response (pCR) in patients receiving neoadjuvant chemotherapy. In a pooled analysis of 3,771 patients treated with neoadjuvant chemotherapy across six German Breast Group trials, increasing TIL concentrations were significantly associated with higher pCR rates in TNBC, with each 10% increment in TILs independently predicting improved disease-free survival (7). The phase 2 NeoPACT trial, which evaluated an anthracycline-free neoadjuvant regimen of pembrolizumab combined with carboplatin and docetaxel, reported pCR rates of 75% in patients with high immune infiltration (TILs ≥30%) compared to 42% among those with low TILs (P = 0.001) (8). Similarly, in the Neo-N trial, pCR rates of 75% were observed with nivolumab plus carboplatin and paclitaxel among patients with both TILs ≥30% and PD-L1 positive compared to pCR rates of 30% among those with both biomarkers negative (9).

Despite the clear relevance of TILs as a prognostic and predictive biomarker, the pivotal KEYNOTE-522 trial did not report outcomes according to TIL levels. In the absence of such data, and recognizing the established importance of this biomarker, the present study was designed to evaluate the impact of baseline TILs on pCR and EFS in patients with early-stage TNBC treated with the KEYNOTE-522 regimen in a real-world setting.

Results

Patients' characteristics

Among 726 patients included in Neo-Real by the time of the analysis, 248 (34.2%) patients had baseline TILs available and were included in this study. The majority had TILs <30% (72.6%),

while 12.9% had TILs in the 30–49% range, and 14.5% had TILs ≥50%. Baseline characteristics between patients included in this TIL analysis and those excluded due to unavailable TIL data are shown in Supplementary Table 1. No statistically significant differences were found in stage distribution, age, or pCR rates between included and excluded patients.

The cohort had a median age of 44 years (range 21–80). Most patients had ductal histology (>91% across all TIL groups) and were premenopausal. The majority (70.8%) had stage II disease. Regarding disease biology, two parameters differed significantly across TIL categories: Ki-67 index ≥50% was more prevalent in the groups with TILs 30–49% (96.9%) and TILs ≥50% (77.8%) compared to the TILs <30% (70.2%) group (P=0.002). Similarly, histological grade 3 disease was more common in the TILs 30–49% (93.7%) and TILs ≥50% (88.2%) groups compared to the TILs <30% group (73.6%; P=0.012). Baseline characteristics according to TIL categories are detailed in Table 1. Clinical T and N status, disease stage, BRCA mutation status, and the choice of AC schedule were balanced across TIL groups. Patterns of surgery, radiotherapy, and adjuvant systemic therapy according to TIL groups are presented in supplementary Table 2.

Pathologic complete response according to TILs levels

Overall pCR rates increased substantially with higher TIL levels, with a statistically significant difference between groups. Patients with TILs <30% had the lowest pCR rates (59% [95% CI 51% - 66.7%]), followed by those with TILs 30–49% (65.6% [95% CI 46.8% - 81.4%]), while patients with TILs ≥50% achieved the highest pCR rates (91.4% [95% CI 77% - 98.2%]) (P=0.001), as illustrated in Figure 1A.

In univariate logistic regression, TILs ≥50% was strongly associated with pCR (OR 7.41, 95% CI 2.18–25.21, P=0.001), while TILs in the 30–49% range did not reach statistical significance compared to TILs <30% (OR 1.33, 95% CI 0.60–2.93, P=0.486). In a complementary analysis

treating TILs as a continuous variable, each 10% increment in stromal TILs was independently associated with a 1.27-fold increase in the odds of achieving pCR (OR 1.27, 95% CI 1.09–1.48; P=0.002). Higher Ki-67 index (≥20%) was also independently associated with pCR (OR 3.32, 95% CI 1.78–6.19, P<0.001). Histological grade 3 and fewer pembrolizumab cycles showed borderline associations in univariate analysis (Table 2).

In the multivariable logistic regression model, TILs ≥50% (OR 6.96, 95% CI 1.89–25.65, P=0.004) and Ki-67 ≥20% (OR 4.89, 95% CI 2.31–10.33, P<0.001) remained independent predictors of pCR. Among patients in the two Ki-67 subgroups, those with TILs ≥50% showed notably elevated pCR rates compared to patients with TILs <50% (Figure 1B and 1C). Of note, virtually all patients (n=26/27, 96.3% [95% CI 81% - 99.9%]) with concurrently high TILs (≥50%) and high Ki-67 (≥50%) achieved a pCR, highlighting the combined predictive value of these two biomarkers.

To address concerns regarding model stability given the modest number of patients with TILs ≥50% (n=36), we performed a sensitivity analysis using Firth's penalized logistic regression, which corrects for small-sample bias and separation. TILs ≥50% remained independently associated with pCR (Firth-penalized OR 5.91, 95% CI 1.75–19.99, P=0.004), closely consistent with the standard multivariable model (OR 6.96, 95% CI 1.89–25.65), supporting the robustness of this finding despite the limited subgroup size.

In an evaluation of RCB 0-1 rates, the results were similar with higher RCB 0-1 rates among patients with TILs ≥50% in the overall cohort and in the cohorts according to Ki67 index. All patients (n=27/27, 100%) with TILs ≥50% and Ki67 ≥50% achieved an RCB 0-1 (Figure 2).

Event-free survival according to TILs levels

With a median follow-up of 24 months, 27 events occurred. The 2-year EFS among patients who achieved pCR was uniformly high across TIL levels, with no statistically significant differences: 97.0% in TILs <30%, 95.2% in TILs 30–49%, and 93.2% in TILs ≥50% (log-rank P=0.828; Figure 3A). These findings suggest that once pCR is achieved, long-term outcomes are excellent regardless of baseline TIL status.

Among patients with residual disease, there was a numerical trend toward better outcomes with higher TIL levels, although this did not reach statistical significance: 2-year EFS was 73.3% in TILs <30%, 90.0% in TILs 30–49%, and 100% in TILs ≥50% (log-rank P=0.350; Figure 3B). Notably, as most patients with TILs ≥50% achieved pCR, only three patients in this subgroup had residual disease; none experienced disease recurrence during follow-up.

In univariate Cox regression, clinical stage III (HR 4.90, 95% CI 2.17–11.03, P<0.001), histological grade 3 (HR 0.41, 95% CI 0.19–0.89, P=0.025), and TILs as a continuous variable (per 10% increment, HR 0.76, 95% CI 0.59–0.98, P=0.041) were significantly associated with EFS (Table 3). In the multivariable Cox model, including TILs as categorical variable, clinical stage and grade, only the clinical stage III (HR 4.65, 95% CI 2.04–10.56, P<0.001) emerged as an independent predictor of EFS. In the multivariable model, TILs were included as a categorical variable (TILs <30%, 30–49%, and ≥50%) rather than as a continuous measure, reflecting the pragmatic clinical applicability of predefined thresholds with well-documented interobserver reproducibility; only clinical stage III emerged as an independent predictor of EFS in this model (HR 4.65, 95% CI 2.04–10.56, P<0.001). Sensitivity analysis including TILs as a continuous variable in the multivariable Cox model did not change this conclusion, with clinical stage III remaining the only independent predictor of EFS.

Discussion

In this real-world cohort of patients with early-stage TNBC treated with the KEYNOTE-522 regimen, high baseline stromal TILs were independently associated with a significantly higher probability of achieving pCR, with an odds ratio of approximately 7 for TILs ≥50% compared to TILs <30%. High TILs were more commonly observed in patients with proliferative tumors, being associated with grade 3 tumors and high Ki-67, in accordance with previous literature (12–14). Notably, high Ki-67 proliferation index was also predictive of pCR. When both biomarkers were simultaneously elevated, the probability of achieving pCR approached 100%, underscoring the clinical value of integrating immune and proliferative biomarkers for treatment response prediction. Our finding are consistent with previous literature on the role of TILs as predictor of pCR in TNBC (7–9) and extend them to the specific context of the KEYNOTE-522 regimen in a real-world Latin American population.

These findings lead to the rationale for neoadjuvant treatment tailoring based on TILs. The BELLINI trial, a notable phase 2 adaptive platform trial, explored a different angle by investigating whether immunotherapy without chemotherapy could achieve meaningful responses in TIL-selected patients. In cohorts specifically selecting patients with TILs ≥50% who were treated with short-course combination immunotherapy achieved pCR rates of 33% with nivolumab/ipilimumab and 47% with nivolumab/relatlimab, without any chemotherapy (15). These findings highlight the concept that, in highly immune-infiltrated tumors, immunotherapy alone may be sufficient to induce meaningful pathologic responses, raising the hypothesis that chemotherapy de-escalation may be feasible in this subgroup; however, the present study, in which all patients received chemoimmunotherapy, cannot address this question directly. Nevertheless, based on the previous mentioned observations of excellent outcomes in chemotherapy-naive patients with high TILs (4–6), an open question is whether some patients could safely forgo systemic therapy altogether.

An important unmet need highlighted by our findings is the absence of TIL-stratified data from the KEYNOTE-522 trial itself. In an exploratory biomarker analysis of the KEYNOTE-522 study, other immune-related biomarkers have been evaluated. The T-cell–inflamed 18-gene expression profile (TcellinfGEP) was able to identify patients with improved pCR and EFS with or without pembrolizumab, confirming that an immune biomarker is able to identify patients with improved outcomes, although TcellinfGEP was not predictive of benefit of immunotherapy (16). Nevertheless, given that TILs are a low-cost, easily available, and well-validated biomarker for treatment response in early-stage TNBC, reporting of outcomes stratified by TIL levels in this pivotal trial is highly warranted. Such an analysis could clarify whether TILs modulate the magnitude of benefit derived from immunotherapy versus chemotherapy and whether certain patient subgroups—particularly those with very high TILs—might achieve equivalent outcomes with less intensive regimens. Our results allow the conclusion that TILs are a predictive biomarker of response to the KEYNOTE-522 regimen but do not establish whether patients with high TILs would achieve equivalent outcomes with less intensive treatment. Recognizing the differences between real-world and clinical trial data, and the absence of a non-pembrolizumab comparator arm in the present study, presentation of efficacy data stratified by TIL levels by the KEYNOTE-522 sponsor and investigators would be highly valuable for guiding future treatment selection strategies.

Several ongoing clinical trials are prospectively testing this possibility. The ETNA trial (NCT 06078384) and the OPTImaL trial (NCT 06476119) are evaluating whether patients with stage I TNBC and high TILs can safely omit chemotherapy. Future studies should explore the integration of static tissue biomarkers with dynamic markers such as radiologic response and circulating tumor DNA (ctDNA). The NeoTRACT trial (NCT 05645380) is exploring TIL- and response-guided

adaptation of NAC, allowing potential omission of anthracyclines in highly immune-infiltrated tumors. Similarly, in the ongoing DespaTIL trial, patients with T1cN0 (with any TILs levels) or T2N0 with high TILs will be directed for tailored neoadjuvant therapy based on radiologic response to potentially omit anthracyclines and immunotherapy for those with complete response after four cycles of carboplatin and taxane (NCT 07074106). The on-going SAFE-DE study is including patients with HER2+ breast cancer or TNBC with stage I disease and high TILs and selecting patients to receive or not adjuvant chemotherapy depending on the post-operative ctDNA positivity (NCT 05058183). Together, these studies aim to use TIL quantification as the principal criterion for treatment personalization in early-stage TNBC. Importantly, the combination of TILs and ctDNA is likely to be more informative than either biomarker alone: while TILs reflect the pre-treatment immune microenvironment, ctDNA captures the residual tumor burden after therapy, and their joint assessment may define subgroups with sufficiently low risk to support safe treatment de-escalation or omission.

Beyond TILs, our data also highlight the clinical utility of Ki-67 as a complementary predictive biomarker. The combination of TILs ≥50% and Ki-67 ≥50% identified a subgroup with near-complete pCR rates. Notably, data from the European GAMBIT network, a multicenter real world study of patients with TNBC treated with neoadjuvant therapy, similarly demonstrated that the combined assessment of Ki-67 and TILs provided the strongest prediction of pCR in TNBC, further corroborating the clinical relevance of integrating immune and proliferative biomarkers across independent cohorts (17). In addition, a pooled analysis of nine GBG/AGO-B neoadjuvant trials presented at the San Antonio Breast Cancer Symposium 2025 (18) demonstrated that the combined assessment of Ki-67 and TILs in residual disease after neoadjuvant chemotherapy provides strong prognostic stratification, with 5-year OS of 92.1% for Ki-67 ≤15% and TILs ≥50%

versus 48% for Ki-67 >15% and TILs ≤10%. This finding extends the relevance of the Ki-67/TIL combination beyond the pre-treatment setting and supports its use in guiding post-neoadjuvant treatment prognostication. Of note, the impact of Ki-67 in the outcome differs depending on the setting – high Ki-67 in the pre-treatment sample emerges as a predictor of higher pCR as shown in our cohort, while high Ki-67 in the post-treatment sample seems to be a predictor of worse long-term outcomes.

Finally, we observed a linear relationship between TILs and pCR: the odds of achieving pCR increased as TIL levels rose when TILs were evaluated as a continuous variable, consistent with findings from a pooled analysis of trials by the German Breast Cancer Group (7). This exploratory finding suggests a broadly graded relationship between TILs and outcomes, supporting the notion that specific cutoffs reflect pragmatic considerations for reproducibility rather than sharp biological inflection points.

Regarding EFS, the current analysis is limited by the low number of events, which restricts the power to detect differences in EFS by TIL subgroup. Nevertheless, the numerical patterns are clinically informative: after achieving pCR, outcomes were uniformly favorable across all TIL levels, suggesting that the beneficial effect of TILs on EFS may be largely mediated through higher pCR rates in the setting of chemoimmunotherapy. Among patients with residual disease, TILs ≥50% was associated with numerically better outcomes, and none of the three patients with residual disease and high TILs experienced recurrence. This intriguing finding suggests that TILs may stratify prognosis among non-responders, consistent with data from the GBG/AGO-B pooled analysis highlighting the value of post-neoadjuvant TIL assessment for risk stratification (18). Nevertheless, due to the low number of patients in the category of residual disease and high TILs,

this observation is hypothesis-generating and should be confirmed in larger prospective datasets with longer follow-up and more mature event rates.

Several strengths and limitations of this study should be noted. Strengths include the real-world, multicenter design across 30 institutions distributed across two Latin American countries, the use of standardized TIL assessment according to international guidelines, and the availability of detailed clinicopathologic and outcome data. Limitations include the retrospective nature of the study, the absence of central pathology review for TIL assessment, and the potential selection bias related to TIL reporting practices across institutions. No formal harmonization exercise, inter-rater reliability testing, or site audit was performed for TIL evaluation. Interobserver variability—particularly near the 30% and 50% thresholds—may have introduced some misclassification and potentially attenuated the observed associations. Ki-67 assessment was also performed locally at each institution without centralized harmonization, which may have introduced inter-institutional variability; however, the use of dichotomized thresholds is expected to mitigate the impact of minor scoring differences. The Neo-Real study team is currently working on obtaining representative tumor material for centralized TIL review and assessment of additional biomarkers in a subset of patients, which might help mitigate these limitations. Another limitation was the small number of events in the EFS analyses, particularly in the high-TIL subgroups. Thus, EFS analyses require more patients and events for maturity and will be repeated in a future Neo-Real analysis.

In conclusion, in this real-world cohort of patients with early-stage TNBC treated with the KEYNOTE-522 regimen, high baseline TILs—particularly in combination with a high Ki-67 index—were strongly associated with pCR. These findings reinforce the biological and clinical relevance of TILs as a predictive biomarker in the immunotherapy era. The trend toward better

EFS with higher TILs among patients with residual disease warrants further investigation with longer follow-up. Reporting of TIL-stratified outcomes from KEYNOTE-522 and ongoing TIL-guided de-escalation trials are essential to define the optimal use of TILs in treatment decision-making for early-stage TNBC.

Methods

Study design and participants

The Neo-Real / GBECAM-0123 study is a retrospective multicenter cohort enrolling patients with early-stage TNBC (stage II–III) treated with pembrolizumab plus neoadjuvant chemotherapy (NAC) across fourteen institutions in Brazil and sixteen in Argentina since 2020. For the present analysis, patients were included if stromal TIL assessment was available in their pathology report and had been conducted according to the International TILs Working Group standardized reporting criteria (10). Any histological subtype could be included.

Baseline stromal TILs from pre-treatment core biopsies were categorized as: <30%, 30–49%, or ≥50%. These thresholds were defined based on prior literature supporting clinically relevant cutoffs (5,7) and an earlier evaluation of Neo-Real data examining the accuracy of different TIL cutoffs for predicting pCR in patients treated with the KEYNOTE-522 regimen (conference abstract, unpublished data) (11). Ki-67 scoring was performed locally at each participating institution using immunohistochemistry according to standard clinical practice and categorized as < 50% or ≥50%.

Electronic medical records were reviewed to collect baseline clinical and pathologic characteristics, details of the treatment received, and outcomes. Data were collected using a centralized REDCap platform. The study was approved by the local ethics committees of each

participating center (protocol approval number 67950623.0.1001.0068 in Brazil and 12805 in Argentina). Given the retrospective nature of the study, a waiver of informed consent was granted for patients who could not be contacted or were deceased. The study was conducted in accordance with the Declaration of Helsinki, with patient data maintained in strict confidentiality. The study is coordinated by the Instituto D'Or de Pesquisa e Ensino (IDOR) and supported by the Grupo Brasileiro de Estudos em Câncer de Mama (GBECAM).

Endpoints and statistical analysis

The primary endpoint was the pCR rate (defined as the absence of residual invasive carcinoma in the breast and axillary lymph nodes, ypT0/Tis ypN0) according to TIL levels. Secondary endpoints included residual cancer burden (RCB) according to TIL levels, EFS according to TIL levels among patients who achieved pCR, and EFS according to TIL levels among patients with residual disease.

Comparisons of categorical variables, including pCR, between groups were performed using Fisher's exact test. The Mann–Whitney test was used to compare continuous variables.

Survival analyses were estimated using the Kaplan-Meier method, with log-rank tests used to compare survival curves. Factors associated with pCR and EFS were evaluated using univariate and multivariable logistic and Cox regression models, respectively. In complementary analyses, stromal TILs were also evaluated as a continuous variable (per 10% increment) in both models to assess the linearity of the association with pCR and EFS. Variables were selected for multivariable models based on statistical significance in univariate analysis ($p \leq 0.10$) or recognized clinical relevance. No imputation method was used. The events-per-variable (EPV) ratio was 22 for the multivariable logistic regression and 6 for the multivariable Cox regression, the latter suggesting potential model instability in the evaluation of factors associated with EFS. All analyses were

performed using Stata software version 15.1 (StataCorp, Texas, USA). A two-sided p-value of <0.05 was considered statistically significant.

Declaration Statements

Data availability: The datasets generated and analyzed during the current study are not publicly available due to institutional data protection policies that restrict external distribution of proprietary clinical databases but are available from the corresponding author on reasonable request.

Code availability: This study did not use any custom code or algorithm. All statistical analyses were performed using standard functions in Stata software version 15.1 (StataCorp, USA).

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Author Contributions

RCB, MCT, JB, DDR, DAS, DMG, CHA, BMZ, AF, MLB, PMH, LT, and RBS contributed to the study conception and design. FW, MOA, RCB, IMS, FCB, MMFM, CPP, VL, MGF, FM, RDPF, CLS, ZSS, DMG, CHA, BMZ, AF, MLB, PMH, LT, NJBG, NCCN, MCG, AMUG, MT, MRM, GR, SS, MPED and RBS contributed to the conduct or collection, data analysis and interpretation. RCB, MOA, NCCN, MCG, and RBS contributed to the drafting of the manuscript and critical revisions. All authors gave their final approval of the manuscript to be submitted.

Competing Interests:

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TABLES AND FIGURES

Table 1. Patients' characteristics according to TILs levels.

	TILs < 30% (N=180)	**TILs 30–49% (N=32)**	**TILs ≥ 50% (N=36)**	**P-value**
Age, median (range)	45 (21–80)	42 (24–74)	41 (27–68)	0.110
Histology				0.975
Ductal	165 (91.7%)	31 (96.9%)	34 (94.4%)	
Special type*	15 (7.3%)	1 (3.1%)	2 (5.6%)	
Premenopausal	96 (54.5%)	21 (65.6%)	24 (66.7%)	0.283
Ki-67 index ≥ 50%	125 (70.2%)	31 (96.9%)	28 (77.8%)	0.002
Grade				0.012
1–2	47 (26.4%)	2 (6.2%)	4 (11.8%)	
3	131 (73.6%)	30 (93.7%)	30 (88.2%)	
Clinical T status				0.642
T1	16 (8.9%)	3 (9.4%)	5 (13.9%)	
T2	112 (62.6%)	23 (71.9%)	26 (72.2%)	
T3	37 (20.7%)	5 (15.6%)	4 (11.1%)	
T4	14 (7.8%)	1 (3.1%)	1 (2.8%)	
Clinical N status				0.933
N0	85 (47.7%)	14 (43.7%)	19 (52.8%)	
N1	58 (32.6%)	13 (40.6%)	12 (33.3%)	
N2	20 (11.2%)	3 (9.4%)	4 (11.1%)	
N3	15 (8.4%)	2 (6.2%)	1 (2.8%)	
Stage				0.321
II	119 (68.0%)	24 (77.4%)	27 (79.4%)	
III	56 (32.0%)	7 (22.6%)	7 (20.6%)	
BRCA status				0.205
Mutated	16 (8.9%)	7 (21.9%)	4 (11.1%)	
Wild-type	130 (72.2%)	22 (68.7%)	24 (66.7%)	
Unknown	34 (18.9%)	3 (9.4%)	8 (22.2%)	

AC schedule				0.477
Every 3 weeks	77 (43.3%)	10 (32.3%)	13 (37.1%)	
Dose-dense	101 (56.7%)	21 (67.7%)	22 (62.9%)	

*Special histological types included metaplastic (n=6), apocrine (n=6), lobular (n=4), micropapillary (n=1), and undifferentiated (n=1).

Table 2. Univariate and multivariable analysis of factors associated with pathologic complete response.

Variable	Univariate Analysis			Multivariable Analysis		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
TILs categories						
TILs < 30% (ref.)	Ref.	–	–	Ref.	–	–
TILs 30% - 49%	1.33	0.60–2.93	0.486	0.84	0.36–2.00	0.700
TILs ≥ 50%	7.41	2.18–25.21	0.001	6.96	1.89–25.65	0.004
TILs continuous						
TILs (per 10% increment)	1.27	1.09-1.48	0.002	–	–	–
Ki-67 index						
Low (< 20%) (ref.)	Ref.	–	–	Ref.	–	–
High (≥ 20%)	3.32	1.78–6.19	<0.001	4.89	2.31–10.33	<0.001
Clinical staging						
Stage II (ref.)	Ref.	–	–	Ref.	–	–
Stage III	0.59	0.32–1.09	0.092	0.60	0.31–1.18	0.138
Histological grade						
Grade I/II (ref.)	Ref.	–	–	Ref.	–	–
Grade III	1.82	0.95–3.50	0.072	1.10	0.50–2.38	0.817
Known BRCA mutation						
No (ref.)	Ref.	–	–	–	–	–
Yes	1.44	0.58–3.62	0.433	–	–	–
Pembrolizumab cycles						
≥ 6 cycles (ref.)	Ref.	–	–	Ref.	–	–
< 6 cycles	0.45	0.22–0.91	0.027	0.51	0.23–1.13	0.099
Dose-dense chemotherapy						
No (ref.)	Ref.	–	–	–	–	–
Yes	1.59	0.91–2.79	0.102	–	–	–
Age						
< 65 years (ref.)	Ref.	–	–	–	–	–
≥ 65 years	0.59	0.28–1.28	0.183	–	–	–

Table 3. Univariate and multivariable analysis of factors associated with event-free survival.

Variable	Univariate Analysis			Multivariable Analysis		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
TILs categories						
TILs < 30% (ref.)	Ref.	–	–	Ref.	–	–
TILs 30% - < 49%	0.42	0.10–1.80	0.244	0.58	0.13–2.57	0.478
TILs ≥ 50%	0.37	0.09–1.55	0.172	0.64	0.14–2.85	0.565
TILs continuous						
TILs (per 10% increment)	0.76	0.59-0.98	0.041	–	–	–
Ki-67 index						
Low (< 20%) (ref.)	Ref.	–	–	–	–	–
High (≥ 20%)	0.62	0.28–1.39	0.243	–	–	–
Clinical staging						
Stage II (ref.)	Ref.	–	–	Ref.	–	–
Stage III	4.90	2.17–11.03	<0.001	4.65	2.04–10.56	<0.001
Histological grade						
Grade I/II (ref.)	Ref.	–	–	–	–	–
Grade III	0.41	0.19–0.89	0.025	0.44	0.19-1.01	0.053
BRCA mutation						
No (ref.)	Ref.	–	–	–	–	–
Yes	0.29	0.04–2.11	0.220	–	–	–
Pembrolizumab cycles						
≥ 6 cycles (ref.)	Ref.	–	–	–	–	–
< 6 cycles	2.18	0.95–5.88	0.064	–	–	–
Dose-dense chemotherapy						
No (ref.)	Ref.	–	–	–	–	–
Yes	1.02	0.46–2.25	0.958	–	–	–
Age						
< 65 years (ref.)	Ref.	–	–	–	–	–
≥ 65 years	1.28	0.44–3.74	0.645	–	–	–

Figure 1. Pathologic complete response (pCR) according to TILs levels in the overall population (A), in patients with Ki-67 index <50% (B), and in patients with Ki-67 index ≥50% (C).

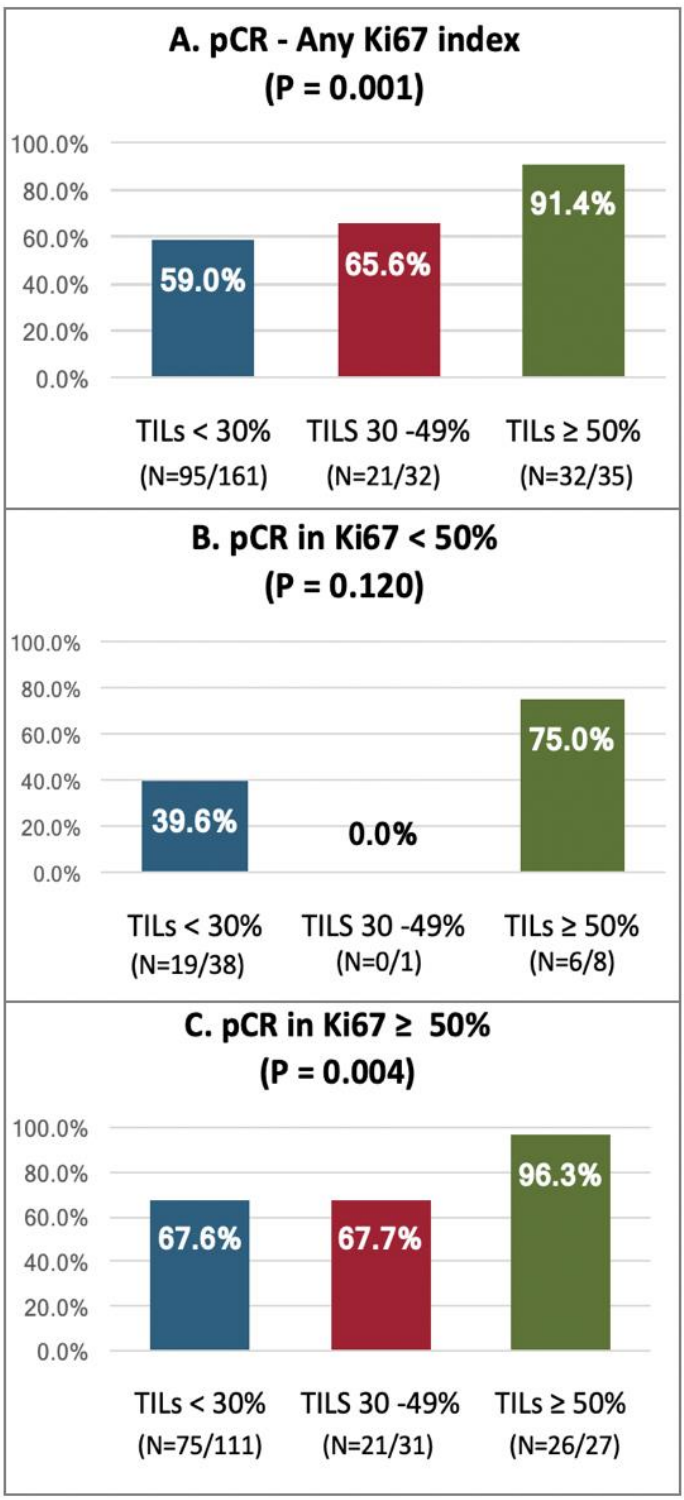

Figure 2. Residual cancer burden (RCB) 0-1 according to TILs levels in the overall population (A), in patients with Ki67 index <50% (B), and in patients with Ki67 index ≥50% (C). Nine patients with residual disease but no RCB classification were conservatively classified as not having RCB 0-1.

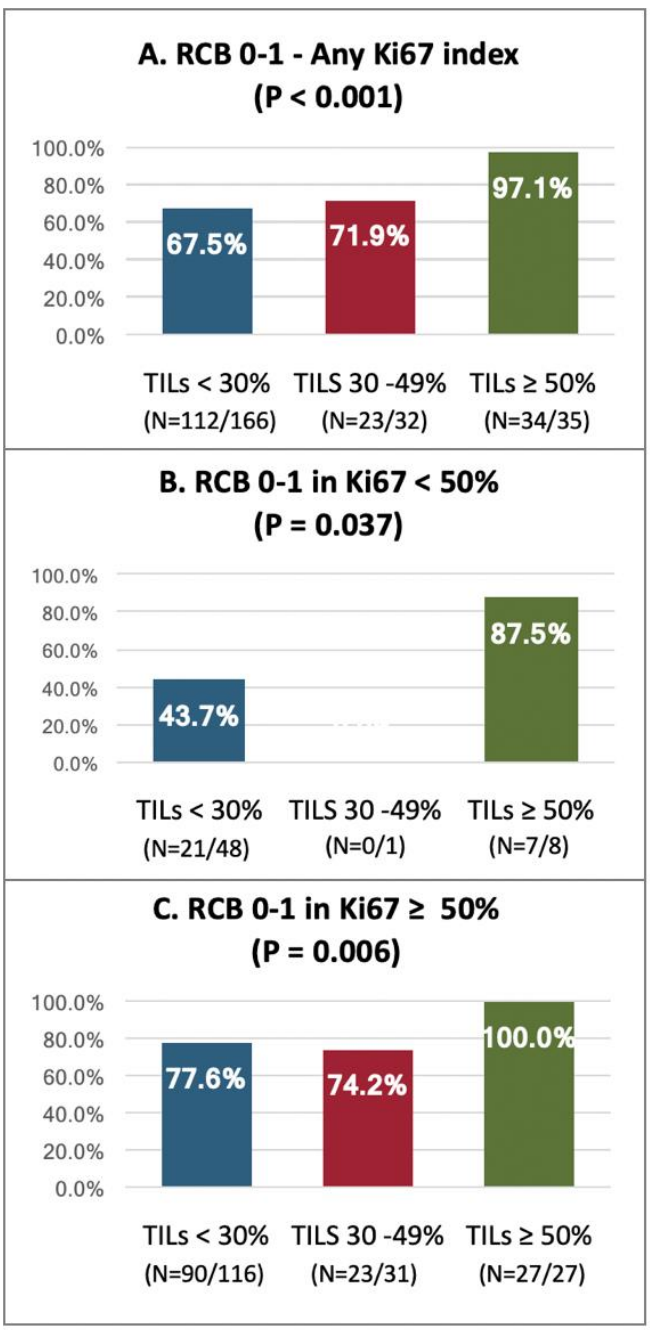

Figure 3. Event-free survival according to TILs levels among patients with pCR (A) and with residual disease (B).