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ORIGINAL ARTICLE

A predictive model of pathologic response based on tumor cellularity and tumor-infiltrating lymphocytes (CelTIL) in HER2-positive breast cancer treated with chemo-free dual HER2 blockade

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Background: The presence of stromal tumor-infiltrating lymphocytes (TILs) is associated with increased pathologic complete response (pCR) and improved outcomes in HER2-positive early-breast cancer (BC) treated with anti-HER2-based chemotherapy. In the absence of chemotherapy, the association of TILs with pCR following anti-HER2 therapy-only is largely unknown.

Patients and methods: The PAMELA neoadjuvant trial treated 151 women with HER2-positive BC with lapatinib and trastuzumab [and hormonal therapy if hormone receptor (HR)-positive] for 18 weeks. Percentage of TILs and tumor cellularity were determined at baseline ($N = 148$) and at day 15 (D15) of treatment ($N = 134$). Associations of TILs and tumor cellularity with pCR in the breast were evaluated. A combined score based on tumor cellularity and TILs (CelTIL) measured at D15 was derived in PAMELA, and validated in D15 samples from 65 patients with HER2-positive disease recruited in the LPT109096 neoadjuvant trial, where anti-HER2 therapy-only was administer for 2 weeks, then standard chemotherapy was added for 24 weeks.

Results: In PAMELA, baseline and D15 TILs were significantly associated with pCR in univariate analysis. In multivariable analysis, D15 TILs, but not baseline TILs, were significantly associated with pCR. At D15, TILs and tumor cellularity were found independently associated with pCR. A combined score (CelTIL) taking into account both variables was derived. CelTIL at D15 as a continuous variable was significantly associated with pCR, and patients with CelTIL-low and CelTIL-high scores had a pCR rate of 0% and 33%, respectively. In LPT109096, CelTIL at D15 was found associated with pCR both as a continuous variable and as group categories using a pre-defined cut-off (75.0% versus 33.3%).

Conclusions: On-treatment TILs, but not baseline TILs, are independently associated with response following anti-HER2 therapy-only. A combined score of TILs and tumor cellularity measured at D15 provides independent predictive information upon completion of neoadjuvant anti-HER2-based therapy.

Clinical trial number: NCT01973660.

Key words: tumor-infiltrating lymphocytes (TILs), HER2, breast cancer, pathologic complete response

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Introduction

Although HER2-positive breast cancer (BC) is a heterogeneous disease, the combination of chemotherapy with trastuzumab-based therapy is considered the standard of care. The benefit of dual HER2-blockade has been well established in advanced BC, where trastuzumab doublets with lapatinib or pertuzumab improves survival [1]. In early-HER2-positive BC, the addition of lapatinib or pertuzumab to trastuzumab-based neoadjuvant chemotherapy increases pathologic complete response (pCR) rates [2, 3]. Moreover, trials investigated dual HER2 inhibition without chemotherapy and demonstrated impressive pCR rates (20%–30%) [2, 4]. Thus, a clinical question that arises is whether dual blockade may eliminate the need for chemotherapy in a subset of patients.

Research focused on stroma tissue and host cells that infiltrate a HER2-positive tumor is getting increasing interest. Indeed, the presence of tumor-infiltrating lymphocytes (TILs) shows high variability across patients and this variability has been associated with patient outcomes. For example, high levels of TILs have been associated with better survival outcome both in the early and metastatic setting [5]. Moreover, TILs have been associated with high-tumor response following neoadjuvant anti-HER2-based chemotherapy [3] and dual HER2 blockade-only with trastuzumab and pertuzumab [2]. However, it is currently unknown if TILs change during anti-HER2 therapy and if this information is associated with clinical outcome.

In this study, we investigated whether early changes in TILs are associated with pathologic response following treatment with lapatinib and trastuzumab in the context of the PAMELA study [4]. In addition, we tried to develop a simple and useful tool to predict response.

Patients and methods

PAMELA study design

The main results of the PAMELA trial have been previously reported [4]. In this study, 151 patients with early-HER2-positive BC were treated with neoadjuvant lapatinib (1000 mg daily) and trastuzumab (8 mg/kg i.v. loading dose followed by 6 mg/kg) for 18 weeks. Patients with hormone receptor (HR)-positive disease received letrozole or tamoxifen according to menopausal status. Formalin-fixed paraffin-embedded (FFPE) tumor samples at baseline and at D15 of treatment were collected according to protocol.

LPT109096 study design

The LPT109096 study results have been previously reported [6]. In this study, 100 patients with early-HER2-positive BC were randomized to lapatinib, trastuzumab or both. Patients received 2 weeks of treatment without chemotherapy, and after a tumor biopsy, they received the same anti-HER2 treatment in combination with chemotherapy consisting of 5FU 500 mg/m^2 + epirubicin 75 mg/m^2 + cyclophosphamide 500 mg/m^2 i.v. every 21 days (FEC75) for four cycles followed by weekly paclitaxel 80 mg/m^2 for 12 weeks. Trastuzumab was administered every week (4 mg/kg i.v. loading dose followed by 2 mg/kg). Lapatinib was administered every day (1250 mg if given without chemotherapy, 750 mg during FEC75 and 1000 mg during paclitaxel). FFPE tumor samples at baseline and at D15 of treatment were collected according to protocol.

TILs and tumor cellularity

Histopathologic analysis of the proportion of TILs was done in whole sections of tumor tissue stained with hematoxylin and eosin (H&E). TILs were quantified according to the 2014 Guidelines developed by the International TILs Working Group [7]. Cases were defined as lymphocyte-predominant BC (LPBC) if TILs represented ≥50%. Percentages of TILs and tumor cellularity at baseline and D15 were scored in slides of core biopsies from patients enrolled in PAMELA and LPT109096 blinded from clinic–pathologic and outcome data.

Tumor cellularity and TILs combined model at D15

From D15 samples of PAMELA, a bivariate logistic regression model for pCR in the breast was carried out using tumor cellularity and TILs as continuous variables. The estimated coefficient of each variable in the logistic model was used to derive an unscaled tumor cellularity and TILs (CelTIL) score = $-0.8 \times$ tumor cellularity (in %) + $1.3 \times$ TILs (in %). The minimum and maximum unscaled CelTIL scores were −80 and 130. This unscaled CelTIL score was then scaled to reflect a range from 0 to 100 points.

Statistical analysis

Association between two variables was evaluated using Student's *t*-test, Pearson's χ^2 test or Fisher's exact test. Association of each variable with pCR was determined by univariate and multivariable logistic regression analysis. pCR was defined as the absence of residual invasive cancer in breast following neoadjuvant therapy (ypT0/is). Odds ratios (OR) with a 95% confidence interval (95% CI) was estimated. Performance of each model was estimated by determining the area under the ROC curve (AUC). All statistical tests were two-sided and considered significant when $P \leq 0.05$. All statistical analyses were carried out using the R software 2.3.0.

Results

Main clinic–pathologic characteristics at baseline

In PAMELA, TILs were successfully evaluated in 148 out of the 151 (98%) baseline samples (Figure 1 and supplementary Table S1, available at *Annals of Oncology* online). The median TIL was 10% and most patients had TILs below 20% (interquartile range 5%–20%). TILs in HR-negative were numerically higher compared with HR-positive disease (median 10% versus 5%; $P = 0.071$). No statistically significant associations were identified between baseline TILs and age, nodal status, menopausal status and tumor stage (supplementary Table S2, available at *Annals of Oncology* online). However, it was significant for histologic grade (HG), where HG 1 showed higher TILs (median 20%) compared with HG 2 (median 5%) or 3 (median 10%). Finally, 12 (8.1%) and 136 (91.9%) baseline tumor samples were classified as LPBC and non-LPBC, respectively.

TILs across the intrinsic subtypes at baseline

TILs varied statistically significantly according to the intrinsic subtype ($P = 0.025$; Figure 2), with the HER2-E subtype showing the highest score (median 10%), followed by the Luminal A (7.5%), Luminal B (5%) and Basal-like (5%). The levels of TILs in HER2-E versus non-HER2-E was statistically significant (median 10% versus 5%, $P = 0.016$). Concordant with this result, 11 out of the 12 LPBC (92%) were identified as HER2-E.

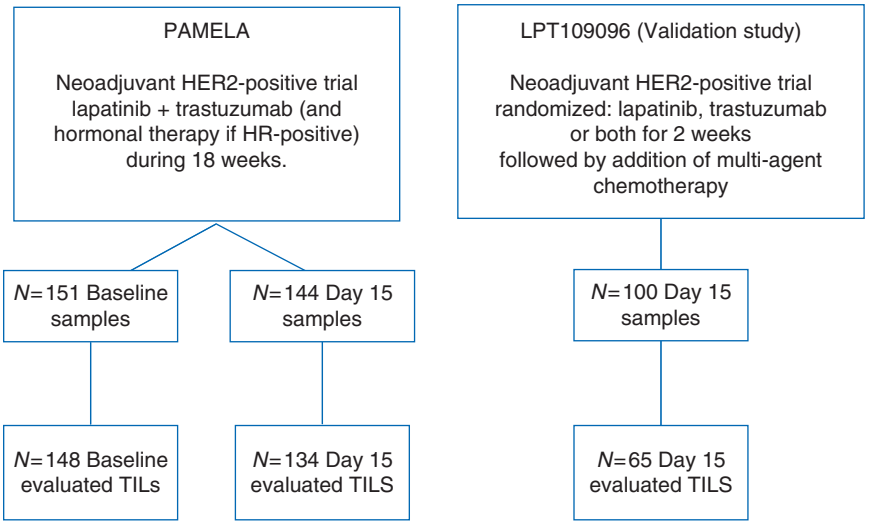


Figure 1. CONSORT diagram.

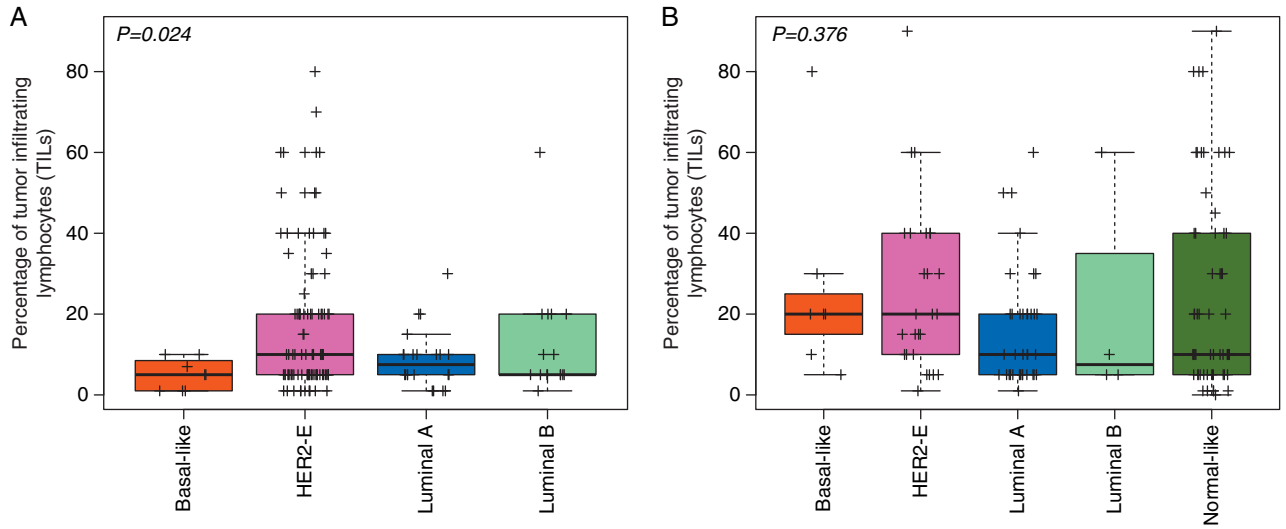


Figure 2. Expression of TILs across the intrinsic molecular subtypes in PAMELA study: (A) at baseline and (B) at day 15.

Association of baseline TILs with pCR

We evaluated the association of baseline clinic–pathologic characteristics and TILs with pCR. In univariate analysis, HG 1, HR-negative status, HER2-E intrinsic subtype and high TILs were statistically significantly associated with pCR (Table 1 and supplementary Figure S1, available at *Annals of Oncology* online). The rates of pCR in LPBC and non-LPBC were 58.3% and 27.2%, respectively (OR = 3.75, 95% CI 1.12–12.54, $P = 0.024$). In multivariable analysis, HG 1, clinically negative nodes and HER2-E subtype were significantly associated with pCR. TILs, however, was not found independently associated with pCR either as a continuous or categorical variable (Table 1).

Changes of TILs at D15

A total of 134 (88.7%) tumor samples at D15 were available, and 131 (86.7%) tumor samples had paired baseline TILs data. Compared with baseline samples, levels of TILs at D15 were statistically significantly higher (mean difference +6.93%, 95% CI 3.28–10.59, $P < 0.001$) (Figure 3). This increase was observed across all subtypes (Figure 2). Concordant with this result, the proportion of LPBC at D15 was higher compared with baseline (8.1% versus 15.7%; $P = 0.048$).

Association of TILs at D15 with pCR

We evaluated the association of baseline clinicopathologic characteristics, TILs and subtype at D15 with pCR. In univariate analysis, HG, HR-negative status, normal-like group, low tumor cellularity and high TILs were statistically significantly associated with pCR (Table 2 and supplementary Figure S2, available at *Annals of Oncology* online). The odds of achieving a pCR increase 4% for every increase of 1% in TILs at D15. The rates of pCR in LPBC and non-LPBC were 65.0% and 21.1%, respectively (OR = 6.96, 95% CI 2.50–18.40, $P < 0.001$). As expected, low % tumor cellularity at D15 was associated with the normal-like phenotype (supplementary Figure S3, available at *Annals of Oncology* online). In multivariable

Table 1. Logistic regression analyses of pathologic complete response including TILs measured at baseline

Variables	*N*	pCR rate (%)	Univariate analysis				Multivariable analysis			
			OR	Lower 95%	Upper 95%	*P*	OR	Lower 95%	Upper 95%	*P*
Age (cont. variable)	–	–	1	0.97	1.02	0.95				
Tumor size (cont. variable)	–	–	1	0.98	1.01	0.18				
Tumor size										
T1	60	30.0	1	–	–	–				
T2	76	31.6	1.08	0.52	2.24	0.84				
T3	12	16.7	0.47	0.10	2.35	0.36				
Menopausal status										
Pre	60	31.7	1	–	–	–				
Post	88	28.4	0.86	0.42	1.75	0.67				
Nodal status										
0	95	33.7	1	–	–	–	1	–	–	–
1–2	53	22.6	0.58	0.27	1.25	0.16	0.36	0.13	0.94	0.04
Histologic grade										
1	22	72.7	1	–	–	–	1	–	–	–
2	27	33.3	0.19	0.05	0.64	0.01	0.24	0.05	0.98	0.05
3	99	19.2	0.09	0.03	0.26	<0.0001	0.10	0.02	0.27	<0.001
HR status										
HR+	71	18.2	1	–	–	–	1	–	–	–
HR-negative	77	42.3	3.30	1.56	6.95	<0.001	1.97	0.78	4.99	0.15
Intrinsic subtype										
Non-HER2-E	48	8.33	1	–	–	–	1	–	–	–
HER2-E	100	40.00	7.33	2.44	22.01	<0.001	6.10	1.69	22.10	0.01
LPBC										
Non-LPBC	136	27.2	1	–	–	–				
LPBC	12	58.3	3.75	1.12	12.54	0.03				
Tumor infiltrating lymphocytes (cont. variable)	–	–	1.02	1.00	1.04	0.03	1.01	0.98	1.03	0.62

OR, odds ratio; pCR, pathologic complete response; HR, hormonal receptor; HER2-E, HER2-enriched; LPBC: lymphocyte-predominant breast cancer.

analysis, HG 1 (versus 3), low tumor cellularity and high TILs were significantly associated with pCR (Table 2). Interestingly, when the association of pCR with baseline TILs, and D15 TILs, as continuous variables, was evaluated in a bivariate model in 131 paired samples, only D15 TILs was found associated with pCR (data not shown). The AUC of tumor cellularity and TILs to predict pCR were 0.793 and 0.695, respectively (Figure 4).

Finally, we explored the absolute change and the ratio from baseline to D15 time points. The absolute change was significantly associated with pCR ($P = 0.004$; AUC = 0.623) as well as the ratio ($P = 0.018$; AUC = 0.597). However, none of these biomarkers was superior to evaluating the levels of TILs at D15 (AUC = 0.695).

Tumor cellularity and TILs combined model at D15 (CelTIL)

The previous analysis suggested that tumor cellularity and TILs at D15 provide independent predictive information from each other. To combine both information into a single variable, the regression coefficients of tumor cellularity and TILs at D15, obtained from the previous multivariable logistic regression model, were used to derive an unscaled CelTIL score [unscaled CelTIL score $= -0.8\times$ tumor cellularity (%) $+ 1.3\times$TILs (%)]. The minimum and maximum unscaled CelTIL scores were −80 and 130, respectively. This unscaled CelTIL score was then scaled to reflect a range from 0 to 100 points.

To further explore the ability of CelTIL to better predict pCR compared with tumor cellularity or TILs at D15, the performance of each individual variable and CelTIL was estimated. As expected, the AUC of CelTIL (0.857) was higher than the AUC of tumor cellularity (0.793) or TILs (0.695). Next, we evaluated the distribution of CelTIL in all D15 samples and observed that it followed a bimodal shaped distribution (Figure 5). Based on the hypothesis that the variable is distributed according to a mixture of two Gaussian distributions, an optimal cut point (scaled CelTIL cut point of 33.59) was identified from the mixture model (Figure 5). Below and above this cut point, the pCR rates were 0% (0/25) and 33% (36/108), respectively (supplementary Table S3, available at *Annals of Oncology* online).

Independent evaluation of CelTIL

A total of 65 (65%) D15 tumor samples from the LPT109096 neoadjuvant HER2-positive trial were evaluated (Figure 1). Patients

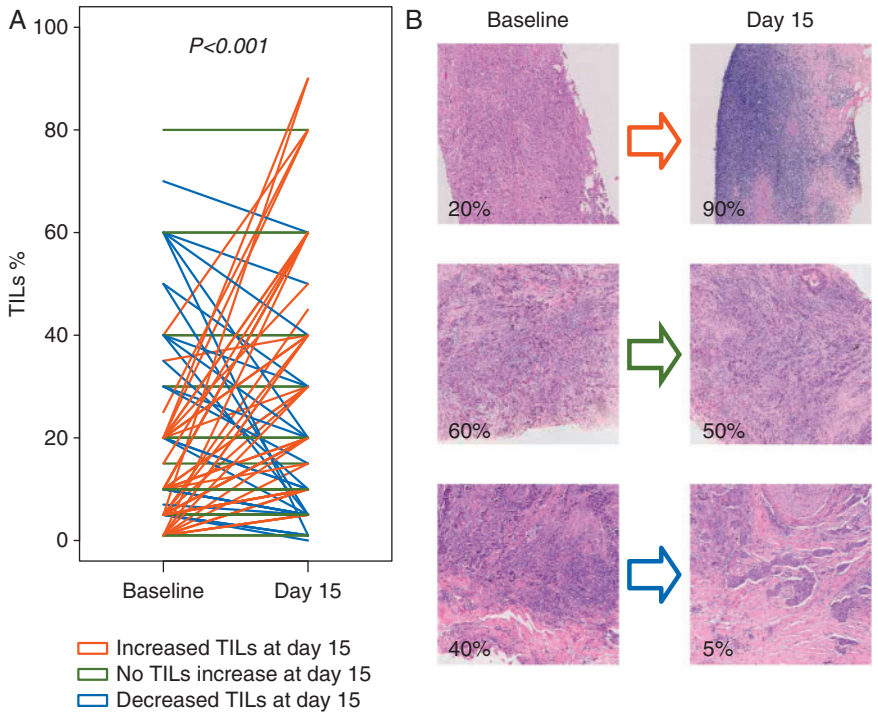


Figure 3. Changes in TILs from baseline to day 15 across 134 cases in PAMELA study: (A) individual changes and (B) microscopic images of three selected cases.

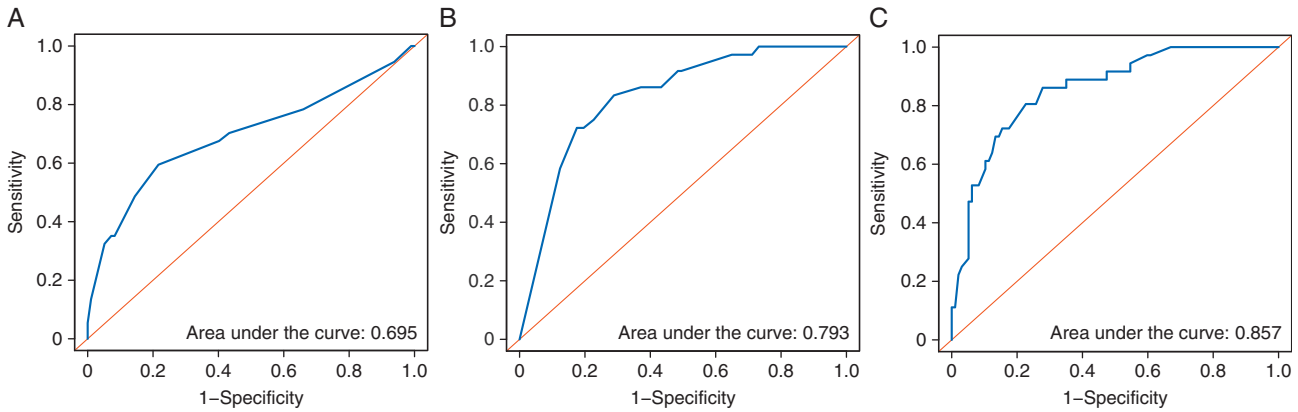


Figure 4. Performance of tumor cellularity or TILs to predict pCR in PAMELA study: (A) TILs measured at day 15; (B) tumor cellularity at day 15 and (C) CelTIL score.

in this study received either lapatinib, trastuzumab or both for 2 weeks, a D15 tumor biopsy was then carried out, followed by the addition of multi-agent chemotherapy for 24 weeks before surgery. As expected, CelTIL was significantly associated with pCR in univariate analysis either as a continuous variable or using the pre-specified cut point (supplementary Table S4, available at *Annals of Oncology* online). Below and above this cut point, the pCR rates were 33.3% (7/21) and 75.0% (33/44), respectively (OR = 6.0, 95% CI 1.93–18.70, $P < 0.001$) (Figure 5). Similar results were obtained when the association of CelTIL with pCR was adjusted for tumor size, nodal status and treatment arm (supplementary Table S4, available at *Annals of Oncology* online). HR status was not found associated with pCR (56.3% in HR-positive versus 66.7% in HR-negative; OR = 0.57, 95% CI 0.57–4.25; $P = 0.390$).

Discussion

Previous studies have revealed that there is a clear relationship between the number of TILs and improved outcomes in patients with HER2-positive BC treated with anti-HER2 agents and chemotherapy. In the NeoALTTO trial, 1% increase in TILs was associated with better event-free survival [3]. Likewise, TILS level greater than 5% was associated with higher pCR rates, independently of treatment. Also, trastuzumab seems to benefit more patients with higher TILs levels, as it has been seen in the FinHER study [8]. In the Tryphaena trial, increase TILs at baseline were associated with increased probability of pCR in univariate but not in the multivariate analysis [9]. In the CherLOB trial, 121 patients were treated with neoadjuvant chemotherapy plus trastuzumab, lapatinib or both. Continuous TILs were associated with

Table 2. Logistic regression analyses of pathologic complete response including TILs and tumor cellularity measured at day 15

Variables	*N*	pCR rate (%)	Univariate analysis				Multivariable analysis			
			OR	Lower 95%	Upper 95%	*P*	OR	Lower 95%	Upper 95%	*P*
Age (cont. variable)	–	–	1	0.97	1.03	0.99				
Tumor size (cont. variable) at baseline	–	–	1	0.99	1.01	0.24				
Tumor size at baseline										
T1	53	28.3	1	–	–	–				
T2	69	29.0	1.03	0.47	2.28	0.84				
T3	12	16.7	0.50	0.10	2.60	0.36				
Menopausal status										
Pre	53	28.3	1	–	–	–				
Post	81	27.2	0.94	0.44	2.04	0.89				
Nodal status at baseline										
0	86	33.7	1	–	–	–	1	–	–	–
1–2	48	21.0	0.58	0.25	1.32	0.09	0.37	0.13	1.11	0.08
Histologic grade at baseline										
1	18	72.2	1	–	–	–	1	–	–	–
2	23	26.1	0.14	0.03	0.53	0.01	0.27	0.05	1.46	0.10
3	93	19.4	0.10	0.03	0.29	<0.0001	0.15	0.04	0.64	0.01
HR status at baseline										
HR+	70	15.7	1	–	–	–	1	–	–	–
HR-negative	64	40.6	3.67	1.63	8.29	<0.01	1.40	0.46	4.20	0.55
Intrinsic subtype at day 15										
Non-normal-like	73	12.3	1	–	–	–	1	–	–	–
Normal-like	60	45.0	5.82	2.46	13.80	<0.001	1.84	0.51	6.61	0.35
LPBC at day 15										
Non-LPBC	114	21.1	1	–	–	–				
LPBC	20	65.0	6.96	2.50	18.40	<0.001				
Tumor infiltrating lymphocytes (cont. variable) at day 15	–	–	1.04	1.02	1.06	<0.001	1.04	1.01	1.06	<0.01
Tumor cellularity (cont. variable) at day 15	–	–	0.93	0.89	0.96	<0.001	0.95	0.91	0.99	0.03

One sample with TIL data has no intrinsic subtype.

OR, odds ratio; pCR, pathologic complete response; HR, hormonal receptor; HER2-E, HER2-enriched; LPBC: lymphocyte-predominant breast cancer.

pCR in the univariate analysis but were not maintained in multivariate analysis when adjusted for intrinsic subtype, similarly as in our study. However, the low sample size should be considered before making definite conclusions [10].

Although all these data demonstrate that TILs at baseline play an important role as predictive and prognostic value, changes in TILs from baseline to D15 have not been previously reported to the best of our knowledge. Based on our data, TILs predictive value is strongest at D15 than baseline. Compared with baseline, week-2 biomarker analyses have been associated with outcomes in a more consistent way in several studies. Farther to the change of ki-67 expression in HR-positive disease following endocrine treatment, a metabolic response by 18F-FDG PET/CT and presenting a normal-like subtype at D15 have been correlated with higher pCR in the NeoALTTO [11] and PAMELA [4] trials, respectively. Indeed, the decrease of the tumor cellularity at D15 was likely to indicate early-tumour response and an increased proportion of normal breast tissue. We have hypothesized that this second biopsy demonstrating a normal-like genomic subtype might capture a lower percentage of tumor cellularity. However, when we have integrated intrinsic subtype, TILs and tumor cellularity in a multivariate analysis, the genomic profiling lost significance, suggesting that not only the number of tumor cells should be considered, but a positive interaction between immune response and tumor cells might be the most potent factors related with pCR. Following these observations, we also developed and validated an easy tool called CelTIL which independently predicts pCR and might potentially select which patients could be cured with the combination of anti-HER2 therapies without chemotherapy. Very similar results were obtained in 65 D15 tumor samples from the LPT109096 trial. Although patients in this study received neoadjuvant chemotherapy, the CelTIL scoring was not affected since during the first 2 weeks, patients received either lapatinib, trastuzumab or the combination.

The finding that a mathematical model that combines data from TILs and tumor cellularity is associated with pCR in HER2-positive BC patients raises several questions. First, does this model correlate with prognosis in other tumor types, such as

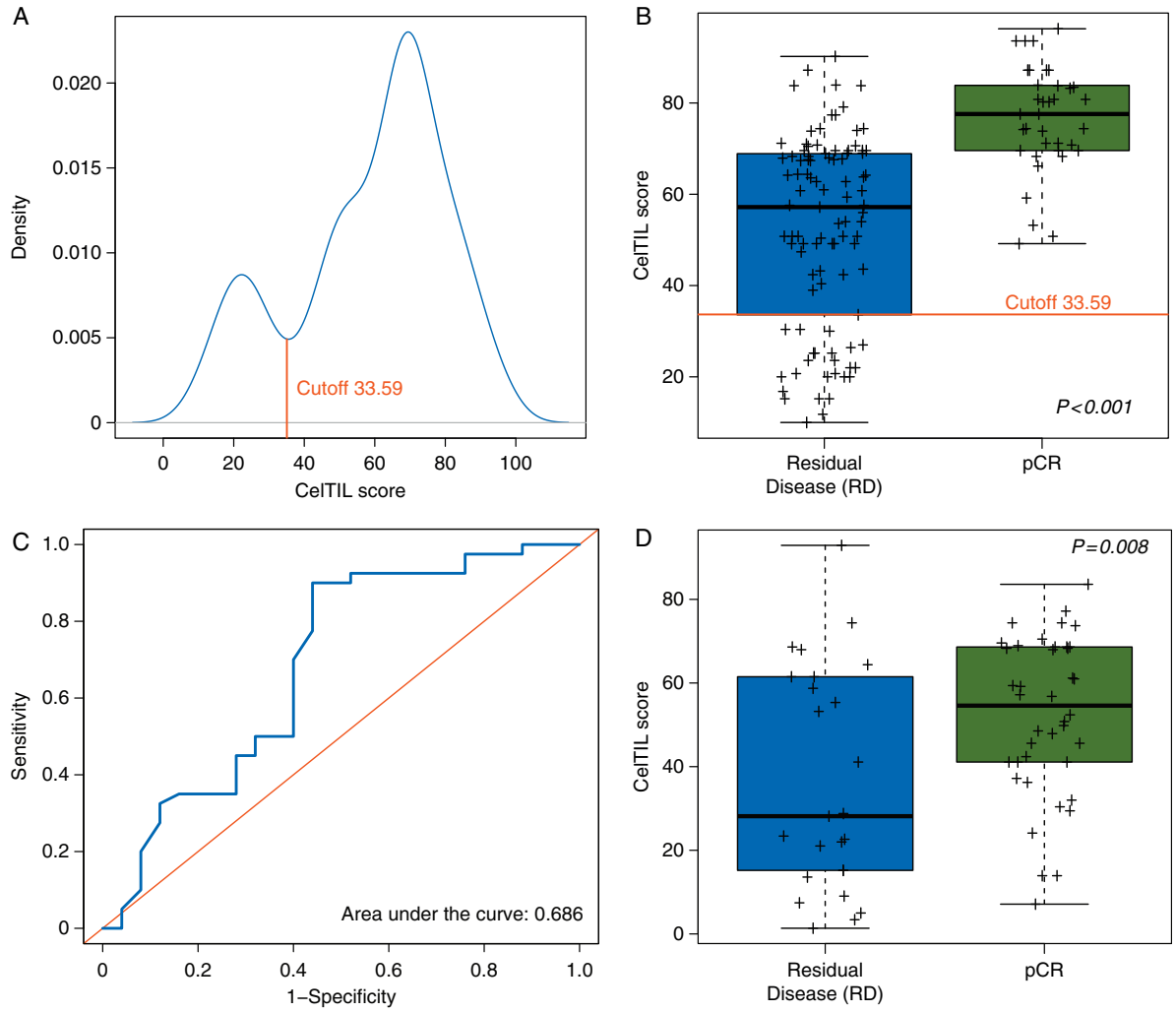


Figure 5. Performance of CelTIL score to predict pCR in PAMELA and LPT109096 studies: (A) density plot of CelTIL in PAMELA, (B) expression of CelTIL in patients achieving a pCR versus residual disease at 18 weeks of treatment in PAMELA, (C) CelTIL score to predict pCR in LPT109096 and (D) CelTIL in patients achieving a pCR versus residual disease in LPT109096.

triple-negative BC as well? Second, does the favorable outcome of patients with higher CelTIL relate to the ability of these patients to respond well to anti-HER2 therapies, or it reflects an intrinsic good prognosis irrespective of treatment? Third, when is the optimal time to define CelTIL? Although the reason for selecting the week-2 biopsy was based on previous studies in HR-positive/HER2-negative disease, time to induce an optimal immune infiltration is unknown. Fourth, is CelTIL an optimal marker for other antiHER2 combinations? Fifth, how does CelTIL correlate with long-term outcomes such as disease-free survival?

Considering that the CelTIL is not scaled per dataset, it can be applied to any new dataset, after calculating TILs and tumor cellularity in a section of FFPE breast tissue with H&E staining. To overcome these issues, we are currently performing a further validation of the predictor in the phase II clinical trial PHERGAIN (NCT03161353), which has started accrual and will evaluate pertuzumab with trastuzumab (and endocrine therapy if HR-positive) without chemotherapy for those patients who achieve a pCR.

In conclusion, our data support the role of CelTIL in selecting patients who have a higher chance of being cured with a chemo-free regimen in HER2-positive early-BC. Although these findings require further validation, they support the concept that stroma tissue interplays with epithelial cells and both, tumor cells and TILs, are integrated into the same immune modulation procedure. Integrating these factors with other classical prognostic and predictive factors should help us better define which patients will be cured without chemotherapy.

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Disclosure

Advisory role of AP for Nanostring Technologies. All remaining authors have declared no conflicts of interest.

References

1. Veeraraghavan J, De Angelis C, Reis-Filho JS et al. De-escalation of treatment in HER2-positive breast cancer: determinants of response and mechanisms of resistance. Breast 2017; 34(Suppl 1): S19–S26.
2. Bianchini G, Pusztai L, Pienkowski T et al. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol 2015; 26: 2429–2436.
3. Salgado R, Denkert C, Campbell C et al. Tumor-infiltrating lymphocytes and associations with pathological complete response and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO Trial. JAMA Oncol 2015; 1(4): 448–454.
4. Llombart-Cussac A, Cortes J, Pare L et al. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. Lancet Oncol 2017; 18(4): 545–554.
5. Luen SJ, Salgado R, Fox S et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol 2017; 18(1): 52–62.
6. Holmes F, Nagarwala Y, Espina V et al. Correlation of molecular effects and pathologic complete response to preoperative lapatinib and trastuzumab, separately and combined prior to neoadjuvant breast cancer chemotherapy. J Clin Oncol 2011; 29(15 Suppl): 506–506.
7. Salgado R, Denkert C, Demaria S et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015; 26(2): 259–271.
8. Loi S, Michiels S, Salgado R et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014; 25(8): 1544–1550.
9. Ignatiadis M, Eynden GGVd, Salgado R et al. Tumor infiltrating lymphocytes before and after dual HER2 blockade in HER2-amplified early breast cancer: A TRYPHAENA substudy. J Clin Oncol 2016; 34: 11507–11507.
10. Dieci MV, Prat A, Tagliafico E et al. Integrated evaluation of PAM50 subtypes and immune modulation of pCR in HER2-positive breast cancer patients treated with chemotherapy and HER2-targeted agents in the CherLOB trial. Ann Oncol 2016; 27(10): 1867–1873.
11. Gebhart G, Gamez C, Holmes E et al. 18F-FDG PET/CT for early prediction of response to neoadjuvant lapatinib, trastuzumab, and their combination in HER2-positive breast cancer: results from Neo-ALTTO. J Nucl Med 2013; 54(11): 1862–1868.