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Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial

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Background: We have previously shown the prognostic importance of tumor-infiltrating lymphocytes (TILs) in newly diagnosed triple-negative breast cancer (TNBC) using tumor samples from a large clinical trial cohort. In this study, we aimed to validate these findings and also investigate associations with trastuzumab benefit in HER2-overexpressing disease (HER2+).

Patients and methods: A prospective–retrospective study was conducted using samples from the FinHER adjuvant, phase III trial that enrolled 1010 early-stage BC patients, 778 of whom were HER2-nonamplified. Those with HER2+ disease ($n = 232$) were randomized to 9 weeks of trastuzumab or no trastuzumab in addition to chemotherapy. Two pathologists independently quantified stromal TILs in 935 (92.6%) available slides. The primary end point of distant disease-free survival (DDFS) and interactions with trastuzumab were studied in Cox regression models.

Results: Confirming our previous findings, in TNBC ($n = 134$) each 10% increase in TILs was significantly associated with decreased distant recurrence in TNBC; for DDFS the hazard ratio adjusted for clinicopathological factors: 0.77; 95% confidence interval (CI) 0.61–0.98, $P = 0.02$. In HER2+ BC ($n = 209$), each 10% increase in lymphocytic infiltration was significantly associated with decreased distant recurrence in patients randomized to the trastuzumab arm (DDFS $P_{interaction} = 0.025$).

Conclusions: Higher levels of TILs present at diagnosis were significantly associated with decreased distant recurrence rates in primary TNBC. These results confirm our previous data and further support that TILs should be considered as a robust prognostic factor in this BC subtype. We also report for the first time an association between higher levels of TILs and increased trastuzumab benefit in HER2+ disease. Further research into why some TN and HER2+ BCs can or cannot generate a host antitumor immune response and how trastuzumab can favorably alter the immune microenvironment is warranted.

Key words: lymphocytic infiltration, breast cancer, prognosis, prediction, biomarkers trastuzumab efficacy, TILs

introduction

Evasion of host immunity is thought to be critical for cancer growth and progression [1]. While breast cancer (BC) has not traditionally been considered an 'immunogenic' tumor type, tumor-infiltrating lymphocytes (TILs) have been correlated with

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a good outcome in a number of cohorts [2–6]. Previously, we have shown by using over 2000 archival BC samples from a prospective clinical trial that higher levels of TILs located in the surrounding stroma were associated with an excellent prognosis but only in the triple-negative (TN) BC subtype. We further postulated that a higher dose of anthracycline in the chemotherapy regimen could promote host antitumor immunity [7]. Interestingly, in BCs that overexpressed the HER2/*neu* oncogene (hereafter known as HER2+), higher levels of TILs were significantly associated with improved survival in patients who received the higher anthracycline dose. This hypothesis was derived from preclinical data demonstrating that the efficacy of some cancer therapies is related to the creation of a favorable immune microenvironment [5, 8].

Trastuzumab, a monoclonal antibody targeted against HER2, has dramatically improved clinical outcomes for women with HER2+ disease. However, despite significant research efforts, no clinically useful biomarkers currently exist that can identify the patients who derive benefit from or are resistant to trastuzumab. This will become an increasingly important issue in an era with a growing array of effective anti-HER2 agents available for clinical use [9–11]. Additional aberrations in the PI3K/AKT, ERK1/2, SRC signaling pathways and loss of PTEN are thought to contribute to resistance but are yet to be validated in the clinical setting [12–14]. However, increasing evidence suggests a significant contribution of innate and adaptive immunity to trastuzumab efficacy [15–19].

The evaluation of prognostic and predictive biomarkers is strengthened by the use of specimens from clinical trial participants [20]. Herein, we conducted 'prospective–retrospective' analysis using tumor samples obtained from the FinHER clinical trial [21] participants to achieve two main objectives. First, we wanted to validate our previous observation that TILs were associated with a good prognosis in triple-negative breast cancer (TNBC) in order to achieve a high level of evidence for the clinical validity of this biomarker. For this analysis, we used the same method of evaluating TILs as previously described [7]. Secondly, as we hypothesized that pre-existing host antitumor immunity would be important for trastuzumab efficacy, we investigated if there was an association between TILs and trastuzumab benefit in HER2+ BCs. For this analysis, the randomization between trastuzumab and no trastuzumab in this clinical trial cohort provides a unique opportunity to identify true predictive biomarkers.

patients and methods

A schema of the original FinHER trial is given in supplementary Figure S1, available at *Annals of Oncology* online [21, 22]. The clinicopathological characteristics of the original cohort and the cohort assessed for TILs are given in Table 1. The Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria were followed for reporting this study [23].

the FinHER study

This study is based on primary breast tumor tissue samples of women diagnosed with high-risk node-negative or node-positive BC and who participated in the FinHER trial ($n = 1010$), a multicenter adjuvant trial sponsored by the Finnish Breast Cancer Group. Eligibility, patient characteristics and results for this study have been previously reported [21, 22]. All women participating were randomly assigned to receive three cycles of docetaxel or vinorelbine, followed by (in both groups) three cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC). The 232 women whose tumors were confirmed to have HER2/*neu* gene amplification were further randomly assigned to receive or not to receive nine weekly trastuzumab infusions. Superiority of the docetaxel and trastuzumab containing arms has been previously reported at the final median follow-up of 62 months [22]. Study participants provided signed informed consents to allow further research analyses to be carried out on their tumor tissue. The ethical committee of the Helsinki University Central Hospital also approved the current study.

pathologic assessment

As per the original protocol, samples were considered hormone receptor positive if ≥10% of cancer cells expressed estrogen and/or progesterone (ER, PR) receptor. Patients with ER- or PR-positive tumor received 5 years of endocrine therapy (tamoxifen and/or aromatase inhibitors). Ki67 immunohistochemistry was assessed locally by IHC using the Mib-1 monoclonal antibody (Dako, Glostrup, Denmark). The number of copies of the *HER2/neu* gene was centrally confirmed by means of chromogenic *in-situ* hybridization (CISH) in one of two reference laboratories. For this study, BC subtypes were classified using IHC as previously published: luminal (ER+ and/or PgR+, HER2−), HER2-positive/overexpressing by CISH (HER2+), and triple negative (TN; ER−, PgR−, and HER2−).

Of the 1010 samples, 935 (92.6%) tumor slides could be retrieved (Table 1). Evaluation of the quantity and location of TILs (stromal and intratumoral) was carried out exactly as previously described on full-face hematoxylin and eosin-stained (H&E) sections [4, 7]. Two pathologists (RS, NS) carried out the readings independently and blinded from clinical outcome with the mean value of two assessments used for the current analyses. The correlation coefficient between the two pathologists was 0.77 ($P < 0.001$) and 0.49 ($P < 0.001$) for stromal and intratumoral TILs, respectively, consistent with our previous findings [7]. Given the low correlation between pathologists for intratumoral TILs, their low variance (median 0.5%, interquartile range 0%–3%, similar to previous observations) only the analyses using stromal TILs are presented.

statistical analysis

In this study, the primary predefined hypothesis was that higher levels of TILs would be associated with a good prognosis in TNBC and trastuzumab benefit in HER2+ BC. Wherever possible, TILs were evaluated as a continuous variable (per increasing 10% increments) [7].

For the survival analyses, the primary end point was distant disease-free survival (DDFS) as defined by the time interval between the date of randomization and the date of first cancer recurrence outside of the ipsilateral locoregional region or to death whenever death occurred before distant recurrence [22]. Patients still alive at the last visit without documented evidence of distant metastases were censored. Overall survival (OS) was defined as the time period from the date of random assignment to the date of death whenever death occurred before distant recurrence; however, there were a small number of events at the final analysis [22]. Associations between TILs and clinicopathological characteristics were investigated with Mann–Whitney *U* test for binary variables and Spearman's rank correlation (ρ) for continuous variables.

A two-sided *P*-value <0.05 was considered significant. Cox proportional hazards regression models were used to test the prognostic value of TILS [hazard ratios and 95% confidence intervals (CI), Wald test]. The possible

Table 1. Difference in cohorts evaluated or not evaluated for TILs compared with the original FinHER cohort

Characteristics	All FinHER cohort ($N = 1010$)	Cohort TILs ($N = 935$)	Cohort no TILs ($N = 75$)	P value between TILs and no TILs cohorts
Mean age (years)	50.9	50.7	50.6	
Range	25–66	25–65	31–64	$P = 0.76$*
Mean tumor size (mm)	26	26.3	24.3	
Range	4–160	6–160	4–70	$P = 0.38$*
Median nodal status	2	2	1.0	
Range	0–32	0–32	0–17	$P = 0.17$*
Histological grade				
I	150 (14.9%)	137 (14.7%)	13 (17.3%)	$P = 0.65$#
II	397 (39.3%)	368 (39.4%)	29 (38.7%)	
III	408 (40.4%)	382 (40.9%)	26 (34.7%)	
Unknown	55 (5.4%)	48 (5.0%)	7 (9.3%)	
ER IHC positive				
Positive	729 (72.2%)	679 (72.6%)	50 (66.7%)	$P = 0.26$#
Negative	280 (27.7%)	255 (27.3%)	24 (33.3%)	
Unknown	1 (0.1%)	1 (0.1%)	1 (0.1%)	
HER2 amplification				
Positive (IHC +3/+2 and FISH+)	231 (22.9%)	209 (22.3%)	22 (29.3%)	$P = 0.16$#
Negative	778 (77%)	725 (77.6%)	53 (70.7%)	
Unknown	1 (0.1%)	1 (0.1%)		
DDFS/OS				
No. of eligible patients	1009[a]	934	75	
No. of DDFS events	163 (16.2%)	154 (16.5%)	9 (12%)	
No. of deaths	94 (9.3%)	91 (9.7%)	3 (4.0%)	
Breast cancer subtype (defined by IHC)				
Luminal (ER+/HER2−)	633 (62.7%)	591 (63.2%)	42 (56%)	$P = 0.35$#
HER2-amplified	231 (22.9%)	209 (22.4%)	22 (29.3%)	
Triple negative (ER−/PR-/HER2−)	145 (14.4%)	134 (14.3%)	11 (14.7%)	
Median follow-up (months)	62	62	62	
HR for trastuzumab benefit				
DDFS (HR + 95% CI), P value	0.64 (0.37–1.11), 0.11	0.58 (0.3–1.03), 0.06	1.6 (0.2–11.5), 0.6	
OS (HR + 95% CI), P value	0.54 (0.27–1.11), 0.09	0.56 (0.27–1.16), 0.12	0.24 (0–3878), 0.5	

[a]One patient was stage IV at diagnosis and excluded from ITT population.
*Mann–Whitney test.
#χ^2 P value.

interaction with trastuzumab treatment was tested using a Wald test after adding a trastuzumab main effect and a product interaction term in the Cox model. All Cox models used a separate baseline hazard for chemotherapy type (docetaxel or vinorelbine). Departures from the proportional hazards assumption were assessed based on the Schoenfeld residuals [24]. A multiviarate Cox proportional hazards modeling was carried out using continuous TILs variable (per 10% increments) and the following clinicopathological characteristics: tumor size [T1 (≤2 cm) versus T2 (>2 cm)], histological grade (1, 2, versus 3), nodal status (negative versus positive), and age (≤50 versus >50 years). In order to test the added prognostic value of continuous TILS to a clinicopathological model, we used the likelihood ratio test. For visualization purposes, the Kaplan–Meier survival curves, defining the groups as high and low TILs according to a predefined variable: lymphocyte-predominant BC phenotype (TILs ≥50%.), were produced [7]. Interaction effects were displayed using forest plots. Analyses were carried out using R software version 2.15.2 (www.R-project.org) and SPSS version 20.0 (Chicago, IL).

results

FinHER baseline patient characteristics

In this study, there were no significant differences in patient characteristics of the TILs series ($n = 935$) when compared with the original series (Table 1). The non-TILs evaluated group had a slightly better OS than those who had TILs evaluated, which was not the case for DDFS (DDFS $P = 0.18$, OS $P = 0.047$).

associations between TILs and clinicopathological characteristics

As expected, higher levels of TILs were significantly associated with ER negativity ($P < 0.001$), HER2 amplification ($P < 0.001$), high histologic grade ($P < 0.001$), more involved lymph-nodes ($P < 0.001$), ductal histology ($P < 0.001$), larger tumors ($P = 0.001$),

and higher Ki67 ($\rho = 0.463$, $P < 0.001$). TILs were higher in the HER2+ and TNBC compared with the luminal BC subtypes ($P < 0.001$, Figure 1A and B).

associations between TILs and prognosis

Consistent with our previous observations, we observed a significant association with a good prognosis in only the TNBC and not in luminal or HER2+ subtypes (Table 2). Multivariate Cox proportional hazards analyses were carried out to investigate the relationship between distant recurrence and other pathological factors in TNBC. TILs as a continuous variable, age, and nodal status were a significant predictors of distant recurrence (Table 3). Each 10% increase in TILs was associated with 13% reduction in the relative risk of distant recurrence (adjusted for clinicopathological factors HR 0.77; 95% CI 0.61–0.98, $P = 0.02$). There was no statistical significance observed for OS likely due to the small number of events observed; however, the point estimate were similar to that for DDFS (adjusted HR 0.81; 95% CI 0.61–1.1, $P = 0.1$) (Table 3).

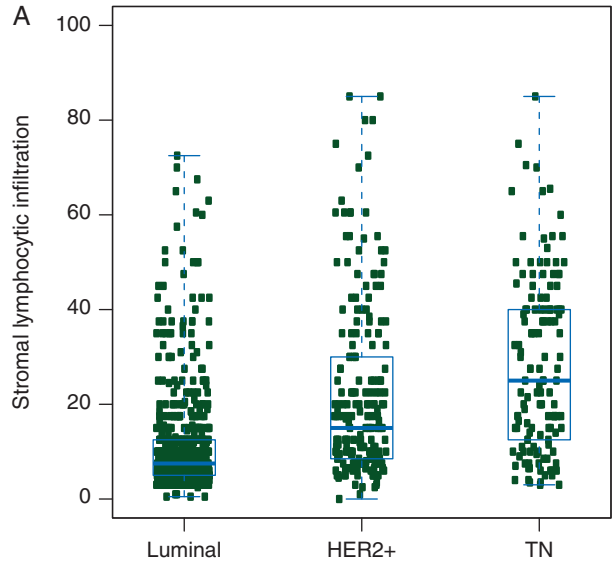
association between TILs and higher trastuzumab benefit

As preclinical data suggest that the host immune system contributes significantly to trastuzumab efficacy, we evaluated the benefit of trastuzumab therapy according to the level of TILs in the HER2+ population [18, 19]. There was a statistically significant interaction between TILs as a continuous variable and trastuzumab treatment ($P_{interaction}$ DDFS $P = 0.025$; $P_{interaction}$ OS $P = 0.08$). For the primary end point of DDFS, each 10% increment in lymphocytic infiltrate was associated with an 18% reduction in the relative risk of distant recurrence in patients who received trastuzumab in addition to their chemotherapy (Figure 2A). These data are visually represented in Figure 2B using a previously defined cutoff (lymphocyte-predominant BC where TILs were present in ≥50% of the tumor [7]). Of note, those who had high TILs at diagnosis and were randomized not to receive trastuzumab seemed to have a worse outcome than those without TILs present.

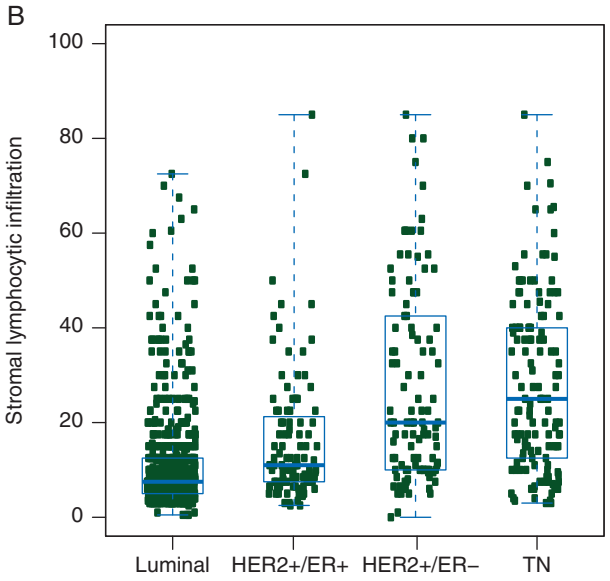
discussion

In this prospectively defined, retrospective study, we report that TILs were significantly associated with prognosis and therapeutic efficacy in the TN and HER2+ BC subtypes, respectively. Notably, we validated our previous observation that increasing lymphocytic infiltration was associated with improved outcomes for patients with TNBC treated with anthracycline-based adjuvant chemotherapy, with remarkably similar adjusted hazard ratio estimates as previously reported [7]. While our finding may not change current chemotherapy options for TNBC patients, this is still a clinically relevant finding as TILs could be useful as stratification or adjustment factor in future clinical studies, as well as providing the rationale for evaluating immunotherapeutic approaches [25].

The potentially most novel finding in this study was the association between TILs and increased benefit from the addition of trastuzumab to chemotherapy in HER2+ disease. This is the first time that an immune biomarker has been shown to be



	Luminal	HER2+	TN
n	591	209	134
Min	0.5	0	3
Q1	5	8.5	12.5
Q2	7.5	15	25
Q3	12.5	30	40
Max	72.5	85	85



	Luminal	HER2+/ER+	HER2+/ER–	TN
n	591	103	106	134
Min	0.5	2.5	0	3
Q1	5	7.5	10	12.5
Q2	7.5	11	20	25
Q3	12.5	21.2	42.5	40
Max	72.5	85	85	85

Figure 1. FinHER dataset: distribution of tumor-infiltrating lymphocytes in breast cancer according to the (A) three breast cancer subtypes and (B) HER2 divided into ER-positive and -negative groups.

Table 2. Univariate prognostic value of stromal tumor-infiltrating lymphocytes (continuous variable per 10% increments) in the global population and breast cancer subtypes from the FinHER dataset for distant disease-free survival (DDFS) and overall survival (OS)

Prognostic value of stromal TILs (per 10% increments)	No. of patients	DDFS[a] HR (95% CI), *P* value	No. of events	OS[a] HR (95% CI), *P* value	No. of events
Global population	934	1.02 (0.92–1.12), 0.73	154	1.07 (0.94–1.2), 0.31	91
Luminal (ER+/HER2−)	591	0.93 (0.74–1.17), 0.55	70	0.99 (0.74–1.34), 0.99	35
HER2+	209	0.96 (0.82–1.3), 0.64	49	0.98 (0.81–1.19), 0.84	31
TNBC	134	0.79 (0.64–0.98), 0.032	35	0.80 (0.62–1.03), 0.08	25

[a]Univariate Cox regression hazard ratios, stratified for chemotherapy type.
HR, hazard ratio; CI, confidence interval.

Table 3. Multivariate Cox proportional analysis of age, nodal status, tumor size, grade, and lymphocytic infiltrate (TILs) in relationship to likelihood of distant recurrence and death in the TNBC subtype from the FinHER dataset

Variables	DDFS *P* value	DDFS HR (95% CI)	OS *P* value	OS HR (95% CI)
Model 1: analysis without stromal TILs variable				
Age, (≤50 versus >50 years)	0.02	0.42 (0.20–0.86)	0.03	0.34 (0.16–0.89)
Nodal status, Positive versus negative	0.02	3.6 (1.22–10.52)	0.13	2.4 (0.77–7.5)
Histologic grade, Grade 3 versus 1/2	0.24	0.62 (0.28–1.14)	0.38	0.66 (0.3–2.8)
Tumor size, T2 (>2 cm) versus T1 (≤2 cm)	0.54	1.2 (0.65–1.35)	0.79	1.13 (0.23–2.8)
Model 2: analysis with stromal TILs variable				
Age, (≤50 versus >50 years)	0.02	0.44 (0.22–0.9)	0.02	0.4 (0.15–0.86)
Nodal status, Positive versus negative	0.045	2.76 (1.02–7.5)	0.19	2.14 (0.68–6.7)
Histologic grade, Grade 3 versus 1/2	0.4	0.71 (0.32–1.57)	0.55	0.75 (0.29–1.93)
Tumor size, T2 (>2 cm) versus T1 (≤2 cm)	0.94	0.97 (0.46–2.046)	0.99	1.01 (0.4–2.53)
Stromal TILs (per 10% increments)	0.02[a]	0.77 (0.61–0.98)	0.14	0.81 (0.61–1.1)

Multivariate model is stratified by chemotherapy type.
[a]Likelihood ratio $P = 0.022$ and χ^2 $(\Delta x^2) = 5.245$ for the comparison of the analysis with and without the stromal TILs variable—i.e. *P* value is based on the value of adding TILs to the model containing clinicopathological factors.
HR, hazard ratio; CI, confidence interval.

predictive of therapeutic benefit, in this case trastuzumab, using samples from a randomized clinical trial. The use of a dataset with this feature significantly strengthens the importance of our findings, in contrast to previous studies using small and single-arm cohorts [17, 26–28].

Thus, although trastuzumab has been thought to act primarily via direct effects on tumor cells, these data highlight the role of antitumor immunity in the efficacy of anti-HER2 treatment. However, if TILs indicate the presence of a tumor-specific immune response, it would seem that the role of immune surveillance for primary tumor control is relatively ineffective as these antigenic primary cancers still can successfully grow. Interestingly, preclinical studies investigating the composition of the T-cell receptor repertoire of TILs suggests an antigenic stimulus from the tumor rather than being nonspecifically induced by the tumor microenvironment [29, 30].

Therefore, to reconcile these findings, we hypothesize that trastuzumab may also serve to relieve suppression of antitumor effector immunity. A similar notion has also been reported in gastrointestinal stromal tumor (GIST) where the efficacy of the tyrosine kinase inhibitor therapy, imatinib, was linked with reduction of tumor expression of *IDO1* [31–33]. Our data also suggest that 'oncogene addiction', in this case HER2 signaling, may be responsible for sustaining an immunosuppressive microenvironment, whereas this seems not be a feature of TNBC. This concept is supported by recent work in mouse models of MYC-driven T-cell acute lymphoblastic lymphoma, BCR-ABL pro B-cell leukemia and BRAF (V600E)-driven melanoma [34–36].

Our study has limitations. While the evaluation of TILs was the prospectively predefined hypothesis for the interaction with trastuzumab therapy, there were only a small number of patients in the HER2+ subgroup and there was a lack of statistically significant effects on OS. This could be related to the small number of events in the final study analysis [22]. Therefore, this trastuzumab-related predictive finding needs additional validation using other datasets. As well as this, further work will be required to increase the analytical validity of the TILs assay before wider clinical implementation, though we note that the correlation between the two independent pathological reviews

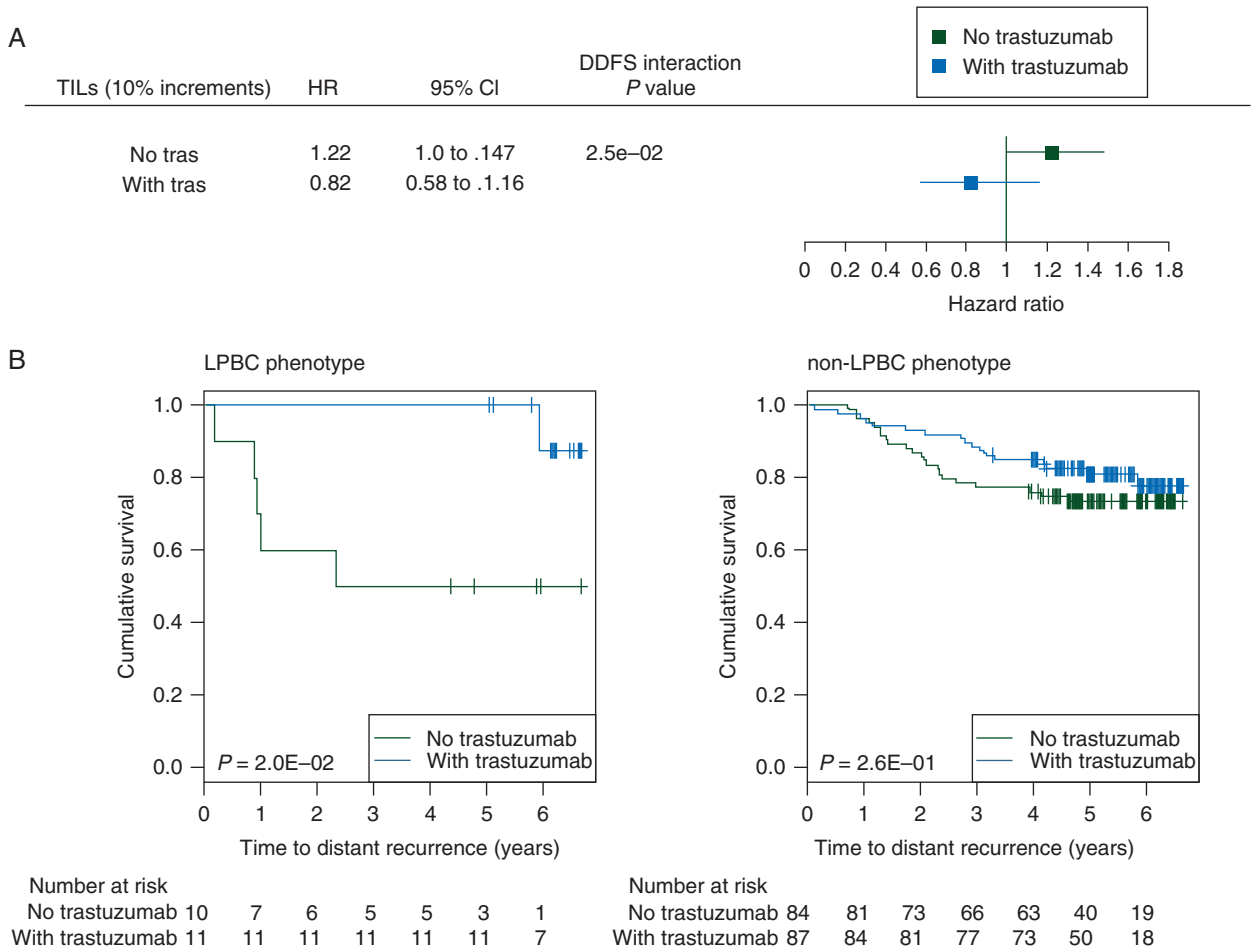


Figure 2. Interactions between stromal tumor-infiltrating lymphocytes (TILs) and trastuzumab benefit in HER2+ disease. (A) Forest plots indicate the prognostic effect of each 10% increment in TILs according to trastuzumab treatment arm. Hazard ratios are derived from Cox regression models stratified by chemotherapy, together with 95% confidence interval (CI) and interaction *P*-value for distant disease-free survival. (B) For illustration, Kaplan–Meier curves comparing trastuzumab versus no trastuzumab for two groups of patients with <50% and >50% levels of stromal TILs (LPBC, lymphocyte predominant breast cancer) at diagnosis are presented (log-rank *P* values are shown).

was high and consistent with our previous work [7]. This work is currently ongoing. In this study, TILs were evaluated using full-face H&E-stained sections, which does make it practical for routine use, in contrast to the use of tissue microarrays [37]. We do not believe that these limitations take away from the significance of our findings. We have also intentionally not indicated a specific cutoff as the relationship between TILs and outcome was found to be continuous (all analyses were conducted with the continuous variable) and we do not want to imply immunophenotypes nor alter treatment decision making at present.

In conclusion, we have confirmed in a prospective–retrospective analysis using samples from the FinHER trial the statistically significant association between increasing lymphocytic infiltration and decreased risk of distant recurrence in high-risk, early-stage TNBC. The use of a second clinical trial dataset to confirm this finding, in addition to further data presented recently in abstract form, suggest that the evidence base for the clinical validity of TILs as a prognostic biomarker in TNBC could now be considered level I [38]. Furthermore, for the first time, we report that level of TILs present at diagnosis could identify the women with primary HER2+ disease who derived higher benefit from the addition of trastuzumab to the anthracyline-based chemotherapy regimen. Our data strongly supports the clinical relevance of antitumor immunity in these two BC subtypes. Further studies will be required to determine how trastuzumab alters the immune microenvironment and if the addition of an immune checkpoint inhibitors can further improve clinical outcomes in these two BC subtypes [39].

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disclosure

The authors have declared no conflicts of interest.

references

1. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331: 1565–1570.
2. Aaltomaa S, Lipponen P, Eskelinen M et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer 1992; 28A: 859–864.
3. Alexe G, Dalgin GS, Scanfeld D et al. High expression of lymphocyte-associated genes in node-negative HER2+ breast cancers correlates with lower recurrence rates. Cancer Res 2007; 67: 10669–10676.
4. Denkert C, Loibl S, Noske A et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010; 28: 105–113.
5. Apetoh L, Ghiringhelli F, Tesniere A et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007; 13: 1050–1059.
6. Desmedt C, Haibe-Kains B, Wirapati P et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res 2008; 14: 5158–5165.
7. Loi S, Sirtaine N, Piette F et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol 2013; 31: 860–867.
8. Mattarollo SR, Loi S, Duret H et al. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res 2011; 71: 4809–4820.
9. Baselga J, Bradbury I, Eidtmann H et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012; 379(9816): 633–640.
10. Baselga J, Cortes J, Kim SB et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012; 366: 109–119.
11. Verma S, Miles D, Gianni L et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012; 367: 1783–1791.
12. Berns K, Horlings HM, Hennessy BT et al. A functional genetic approach identifies the PI3 K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007; 12: 395–402.
13. Nagata Y, Lan KH, Zhou X et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004; 6: 117–127.
14. Zhang S, Huang WC, Li P et al. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. Nat Med 2011; 17: 461–469.
15. Clynes RA, Towers TL, Presta LG et al. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 2000; 6: 443–446.
16. Ignatiadis M, Singhal SK, Desmedt C et al. Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. J Clin Oncol 2012; 30: 1996–2004.
17. Ladoire S, Arnould L, Mignot G et al. T-bet expression in intratumoral lymphoid structures after neoadjuvant trastuzumab plus docetaxel for HER2-overexpressing breast carcinoma predicts survival. Br J Cancer 2011; 105: 366–371.
18. Park S, Jiang Z, Mortenson ED et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 2010; 18: 160–170.
19. Stagg J, Loi S, Divisekera U et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci USA 2011; 108: 7142–7147.
20. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 2009; 101: 1446–1452.
21. Joensuu H, Kellokumpu-Lehtinen PL, Bono P et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 2006; 354: 809–820.
22. Joensuu H, Bono P, Kataja V et al. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer Trial. J Clin Oncol 2009; 27: 5685–5692.
23. McShane LM, Altman DG, Sauerbrei W et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005; 97: 1180–1184.
24. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based weighted residuals. Biometrika 1994; 81: 515–526.
25. Goldhirsch A, Winer EP, Coates AS et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013; 24: 2206–2223.
26. Arnould L, Gelly M, Penault-Llorca F et al. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer 2006; 94: 259–267.
27. Gennari R, Menard S, Fagnoni F et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res 2004; 10: 5650–5655.
28. Ladoire S, Arnould L, Mignot G et al. Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat 2011; 125: 65–72.
29. Sainz-Perez A, Lim A, Lemercier B et al. The T-cell receptor repertoire of tumor-infiltrating regulatory T lymphocytes is skewed toward public sequences. Cancer Res 2012; 72: 3557–3569.
30. Sommerfeldt N, Schutz F, Sohn C et al. The shaping of a polyvalent and highly individual T-cell repertoire in the bone marrow of breast cancer patients. Cancer Res 2006; 66: 8258–8265.
31. Balachandran VP, Cavnar MJ, Zeng S et al. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. Nat Med 2011; 17: 1094–1100.
32. Zitvogel L, Kroemer G. Anticancer effects of imatinib via immunostimulation. Nat Med 2011; 17: 1050–1051.
33. Menard C, Blay JY, Borg C et al. Natural killer cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. Cancer Res 2009; 69: 3563–3569.
34. Knight DA, Ngiow SF, Li M et al. Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. J Clin Invest 2013; 123: 3182.
35. Rakhra K, Bachireddy P, Zabuawala T et al. CD4+ T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. Cancer Cell 2010; 18: 485–498.
36. Khalili JS, Liu S, Rodriguez-Cruz TG et al. Oncogenic BRAF(V600E) promotes stromal cell-mediated immunosuppression via induction of interleukin-1 in melanoma. Clin Cancer Res 2012; 18: 5329–5340.
37. Mahmoud SM, Paish EC, Powe DG et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011; 29: 1949–1955.
38. Adams S, Gray R, Demeria S et al. Prognostic value of tumor-infiltrating lymphocytes (TILs) in two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. San Antonio Breast Cancer Symposium. San Antonio, TX, Abstr S1-07 2013.
39. Loi S, Michiels S, Salgado R et al. Tumor infiltrating lymphocytes (TILs) indicate trastuzumab benefit in early-stage HER2-positive breast cancer (HER2 + BC). San Antonio Breast Cancer Symposium. San Antonio, TX, Abstr S1-05 2013.