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ORIGINAL RESEARCH

Therapeutic Effect of Trastuzumab in Neoadjuvant-Treated HER2-Positive Breast Cancer with Low Infiltrating Level of Tumor-Infiltrating Lymphocytes

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Purpose: The aim of the present study was to investigate the effect of trastuzumab on the pathological complete response (pCR) rate and event-free survival (EFS) in neoadjuvant-treated HER2-positive breast cancer with a low infiltrating level of tumor-infiltrating lymphocytes (TILs).

Patients and Methods: The infiltrating level of TILs was evaluated in hematoxylin and eosin-stained slides from diagnostic needle biopsies, and a low infiltrating level of TILs was defined as TILs < 10%. Data of 179 HER2-positive patients with a low infiltrating level of TILs were retrospectively reviewed and compared according to whether trastuzumab was administered or not. The associations of clinicopathological characteristics with pCR or EFS were assessed in univariate and multivariate analyses. EFS was estimated by the Kaplan–Meier method and compared by the log rank test.

Results: Of 179 patients, the overall pCR rate was 20.1%, and 74 patients (41.3%) received trastuzumab. Patients treated with trastuzumab showed a pCR rate of 20.3% compared with 20.0% for those without trastuzumab ($P = 0.965$). Trastuzumab administration was not associated with pCR in univariate ($P = 0.965$) and multivariate ($P = 0.994$) analyses. Negative status of hormone receptor (HR) ($P < 0.001$) and histological grade 3 ($P = 0.007$) were independent predictors for pCR in multivariate analyses. Trastuzumab usage had no significant impact on EFS in univariate ($P = 0.916$) and multivariate ($P = 0.431$) analyses, and pCR was the only independent predictor for favorable EFS ($P = 0.012$) in multivariate analyses.

Conclusion: In neoadjuvant-treated HER2-positive breast cancer with a low infiltrating level of TILs, additional trastuzumab had no significant influence on the pCR rate and EFS. HR-negative status and histological grade 3 were independently associated with higher pCR rates, and pCR was the only independent predictor for improved survival. Our findings may help identify patients who are resistant to trastuzumab, thereby guiding the de-escalating choice of anti-HER2 therapy.

Keywords: HER2, trastuzumab, tumor-infiltrating lymphocytes, pathological complete response, event-free survival

Introduction

Neoadjuvant treatment (NAT) was initially applied in primary inoperable breast cancer for tumoral shrinkage and surgical feasibility.[1] Increasingly in non-metastatic disease, NAT has been utilized for facilitation of breast conservation,[2] and assessment of treatment response by pathological evaluation of surgical specimens.[3–5] Substantial evidence reveals associations of long-term outcomes with pathological response to NAT,[6–9] and

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pathological complete response (pCR) has been demonstrated as a surrogate indicator for favorable prognosis.[10–12] Furthermore, the survival benefit of postoperative escalating therapy in patients without pCR after NAT has been validated in randomized trials.[13,14] However, the pCR rate and the impact of pCR on survival are highly distinct among molecular subtypes of breast cancer.[10,11,15]

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer represents a distinctive molecular subtype with aggressive biological behavior and poor clinical outcomes.[16–18] Trastuzumab, a recombinant monoclonal antibody that targets HER2, plays a fundamental role in HER2-directed treatment.[19–21] In the neoadjuvant scenario, addition of trastuzumab has significantly increased the pCR rate and improved prognosis compared with chemotherapy alone.[22–25] Moreover, achieving pCR in HER2-positive disease significantly correlates with better survival in terms of event-free survival (EFS) and overall survival.[11,26] Nevertheless, more than half of trastuzumab-treated patients show residual disease after NAT.[10,11] This heterogeneous sensitivity has prompted investigations into the mechanisms of response and resistance to trastuzumab. However, currently, no clinically meaningful biomarkers can identify the patients who will or will not respond to trastuzumab. This issue will be increasingly critical as the efficacy of trastuzumab is fundamental and irreplaceable despite novel anti-HER2 agents.[19–21]

Preclinical evidence suggests a key role of immune mechanisms in modulation of the effect of trastuzumab.[27–29] The antitumor immune response depends on the infiltration of anti- or proinflammatory immune cells in the tumor microenvironment.[27–29] Tumor-infiltrating lymphocytes (TILs), observed as mononuclear immune cells in and around tumor tissue by hematoxylin and eosin (H&E)-stained slides, represent a surrogate marker for antitumor immunity.[30,31] In HER2-positive breast cancer treated with NAT, higher infiltrating levels of TILs have been shown to be associated with higher pCR rates and better survival.[32–36] Contrarily, in spite of treated with various trastuzumab-containing therapies, patients with low infiltrating levels of TILs generally show lower pCR rates and worse prognosis.[32–36] However, little evidence focuses on the benefit of trastuzumab usage in patients with low infiltrating levels of TILs.

The aim of our study was to study the effect of trastuzumab on the pCR rate and EFS in neoadjuvant-treated HER2-positive breast cancer with a low infiltrating level of TILs. TILs were evaluated based on consensual guidelines,[30,37] and data for patients with a low infiltrating level of TILs were categorized and compared according to whether trastuzumab was used or not.

Patients and Methods

Patients

A retrospective review of the database at the breast disease center of our hospital was performed with approval from the Ethics Committee of Sichuan Cancer Hospital & Institute. This study was conducted in compliance with the Declaration of Helsinki. Because of the retrospective nature of our study, patient consent was not required by the Ethics Committee, and the data related to our study were anonymized.

Patients with invasive non-metastatic breast cancer who underwent NAT and subsequent radical surgery from 2008 to 2018 were identified. The inclusion criteria of the present study were female sex, age 18–70 years, histologically confirmed HER2 positivity, completion of all predetermined cycles of standard neoadjuvant chemotherapy, available information of baseline clinicopathological characteristics, definite status of postoperative pathological response, complete data of postoperative treatment and follow-up, available H&E-stained slides of core needle biopsies from primary tumors for TILs assessment, and a low infiltrating level of TILs defined by our preestablished cut-off point of TILs density.

For neoadjuvant chemotherapy, all predesigned cycles had to be completed, and combined or sequential chemotherapy regimens were allowed. Trastuzumab was not administered to all patients for reasons of health insurance and medical cost. In trastuzumab-based NAT, a taxane plus carboplatin (TCb), and an anthracycline plus cyclophosphamide followed by a taxane (AC-T) were regarded as standard chemotherapy. Neoadjuvant trastuzumab had to be used concurrently with taxane, and adjuvant trastuzumab had to be continued to complete 1 year of treatment. For patients who did not receive neoadjuvant trastuzumab, an anthracycline plus cyclophosphamide and a taxane (TAC) and AC-T were considered standard chemotherapy. Patients treated without neoadjuvant trastuzumab but with adjuvant trastuzumab were not included in our study.

Pathological Evaluation

Data for routine pathological parameters were reviewed by experienced breast pathologists. HER2 positivity was defined as either a 3+ score by immunohistochemistry or gene amplification by fluorescent in situ hybridization. Tumor histology was assigned according to the 2012 World Health Organization Classification of Tumors of the Breast. Histological grade was determined by the Scarff–Bloom–Richardson classification

modified by Elston and Ellis.[38] The Ki67 score was defined as the percentage of nuclear stained cells among at least 1000 tumor cells counted, and the median score was used as the cut-off value for statistical analyses. Estrogen receptor (ER) and progesterone receptor (PR) status were considered positive if ≥1% of the cancer cells had nuclear staining.[39] Hormone receptor (HR) status was judged positive if the ER and/or PR status was positive, whereas negative status for both ER and PR was considered HR-negative status. Pathological complete response was defined as no evidence of residual invasive disease in the breast and axilla (ypT0/is ypN0).

Full-face H&E-stained slides of core needle biopsies of primary tumors were utilized for TILs evaluation based on current consensuses standardized by the International Immuno-Oncology Biomarker Working Group.[30,37] All mononuclear cells, including lymphocytes and plasma cells, in the stromal compartment within the tumor border were counted as TILs, and the infiltrating degree of TILs was quantified as the percentage of stromal tissue occupied by TILs. The infiltrating level of TILs was assessed by experienced breast pathologists in a blind manner. A cut-off point of 10% for the TILs percentage was preestablished as a criterion for the inclusion of patients. A low infiltrating level of TILs was defined as TILs < 10% in the present study (Figure 1).

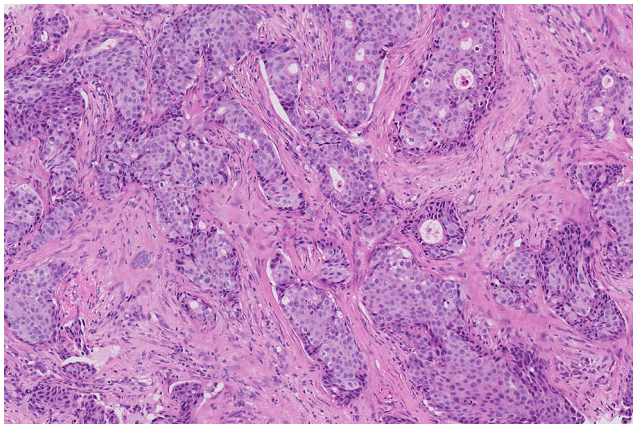

Figure 1 Representative image of a low infiltrating level of tumor-infiltrating lymphocytes.

Statistical Analysis

The chi-square test or Fisher's exact test was conducted for comparisons of categorical variables. Univariate and multivariate logistic regression analyses were performed to assess the associations of pCR with patient and tumor characteristics, and to calculate odds ratios (ORs) and 95% confidence intervals (CIs). For survival analyses, EFS was defined as the interval from the date of diagnosis to the date of events such as locoregional recurrence, distant metastasis, or death from any cause. Univariate and multivariate Cox proportional hazards regression analyses were conducted to evaluate the associations of EFS with patient and tumor characteristics, and to estimate hazard ratios and 95% CIs. The Kaplan–Meier method was employed to draw survival curves, and EFS was compared by the log rank test. P values < 0.05 were considered statistically significant; all tests were two-sided. Statistical analyses were carried out using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) and SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient and Tumor Characteristics

The entire cohort consisted of 179 consecutive HER2-positive breast cancer patients, including 105 patients (58.7%) treated without trastuzumab and 74 patients (41.3%) with trastuzumab. Patient and tumor characteristics are summarized in Table 1. The median age was 53 (range 28–70) years. Most patients were over 50 years (62.0%), and 64.8% of patients were postmenopausal. The majority of tumors had a clinical tumor size of T1–2 (83.8%), and 52.0% of patients had positive nodal status. Histologically, 96.6% of tumors were invasive ductal disease, and most tumors were histological grade 1–2 (67.6%) or had Ki67 score ≤ 35% (53.1%). HR-positive tumors constituted 68.7% of the entire cohort.

As for treatment, 96 patients (53.6%) received sequential neoadjuvant chemotherapy (AC-T), and 83 patients (46.4%) were treated with combined therapy, either TCb or TAC. All chemotherapy regimens were given every 3 weeks for a predefined 6 or 8 cycles. In trastuzumab-containing therapy, a loading dose of 8 mg/kg intravenous trastuzumab followed by 6 mg/kg every 3 weeks was administered for a total of 1 year. The majority of patients underwent mastectomy for the breast (84.4%) or axillary lymph node dissection for the axilla (72.1%). Adjuvant treatment was performed in 66.5% of patients for radiotherapy, and in 65.4% of patients for endocrine therapy, respectively.

Therapeutic Effect of Trastuzumab

All patient and tumor characteristics were compared according to whether trastuzumab was used or not. No significant

Table 1 Patient and Tumor Characteristics

Characteristics	All Patients (n=179)	Without Trastuzumab (n=105)	With Trastuzumab (n=74)	*P* value
Age (Years)				
≤50	68 (38.0%)	40 (38.1%)	28 (37.8%)	
>50	111 (62.0%)	65 (61.9%)	46 (62.2%)	0.972
Menopausal Status				
Pre	63 (35.2%)	36 (34.3%)	27 (36.5%)	
Post	116 (64.8%)	69 (65.7%)	47 (63.5%)	0.761
Clinical Tumor Size[a]				
T1–2	150 (83.8%)	89 (84.8%)	61 (82.4%)	
T3–4	29 (16.2%)	16 (15.2%)	13 (17.6%)	0.677
Clinical Nodal Status[a]				
N0	86 (48.0%)	51 (48.6%)	35 (47.3%)	
N+	93 (52.0%)	54 (51.4%)	39 (52.7%)	0.867
Histology				
Ductal	173 (96.6%)	101 (96.2%)	72 (97.3%)	
Other	6 (3.4%)	4 (3.8%)	2 (2.7%)	0.685
Histological Grade				
3	58 (32.4%)	33 (31.4%)	25 (33.8%)	
1–2	121 (67.6%)	72 (68.6%)	49 (66.2%)	0.740
Ki67 Score				
>35%	84 (46.9%)	47 (44.8%)	37 (50.0%)	
≤35%	95 (53.1%)	58 (55.2%)	37 (50.0%)	0.489
HR Status				
Negative	56 (31.3%)	33 (31.4%)	23 (31.1%)	
Positive	123 (68.7%)	72 (68.6%)	51 (68.9%)	0.961
Neoadjuvant Chemotherapy				
Combined	83 (46.4%)	48 (45.7%)	35 (47.3%)	
Sequential	96 (53.6%)	57 (54.3%)	39 (52.7%)	0.834
Surgery of the Breast				
Mastectomy	151 (84.4%)	89 (84.8%)	62 (83.8%)	
Breast conservation	28 (15.6%)	16 (15.2%)	12 (16.2%)	0.859
Surgery of the Axilla				
SLNB	50 (27.9%)	30 (28.6%)	20 (27.0%)	
ALND	129 (72.1%)	75 (71.4%)	54 (73.0%)	0.821
Adjuvant Radiotherapy				
Yes	119 (66.5%)	68 (64.8%)	51 (68.9%)	
No	60 (33.5%)	37 (35.2%)	23 (31.1%)	0.562
Adjuvant Endocrine Therapy				
Yes	117 (65.4%)	69 (65.7%)	48 (64.9%)	
No	62 (34.6%)	36 (34.3%)	26 (35.1%)	0.906

Note: [a]Clinical tumor size and nodal status were assessed according to the 8th edition of the American Joint Committee on Cancer TNM staging system.
Abbreviations: ALND, axillary lymph node dissection; HR, hormone receptor; SLNB, sentinel lymph node biopsy.

differences were found among patients treated without or with trastuzumab (Table 1). Overall, 36 patients (20.1%) achieved a pCR, and 48 patients (26.8%) had survival events over a median follow-up of 51 months (range 13–126). The associations of trastuzumab administration with pCR or EFS were investigated along with patient and tumor

Table 2 Associations of pCR with Patient and Tumor Characteristics in Univariate and Multivariate Logistic Regression Analyses

Characteristics	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age, years (≤50 vs >50)	1.21	0.58–2.55	0.611	1.29	0.36–4.67	0.698
Menopausal status (pre vs post)	1.05	0.49–2.25	0.898	1.12	0.30–4.22	0.870
Clinical tumor size (T1–2 vs T3–4)	1.25	0.44–3.54	0.674	2.36	0.61–9.15	0.214
Clinical nodal status (N0 vs N+)	1.94	0.92–4.09	0.082	1.59	0.64–3.95	0.319
Histology (ductal vs other)	1.27	0.14–11.21	0.831	0.68	0.06–7.97	0.757
Histological grade (3 vs 1–2)	2.58	1.22–5.44	0.013	3.25	1.39–7.62	0.007
Ki67 score (>35% vs ≤35%)	1.02	0.49–2.11	0.968	1.02	0.44–2.39	0.957
HR status (negative vs positive)	3.72	1.74–7.93	0.001	5.33	2.11–13.45	<0.001
Neoadjuvant chemotherapy (combined vs sequential)	1.20	0.58–2.49	0.625	0.56	0.23–1.37	0.201
Trastuzumab (with vs without)	1.02	0.48–2.14	0.965	1.00	0.45–2.26	0.994

Abbreviations: CI, confidence interval; HR, hormone receptor; OR, odds ratio; pCR, pathological complete response.

characteristics in univariate and multivariate analyses. Trastuzumab had no significant impact on the pCR rate in univariate (OR, 1.02; 95% CI, 0.48–2.14; $P = 0.965$) and multivariate (OR, 1.00; 95% CI, 0.45–2.26; $P = 0.994$) logistic regression analyses (Table 2). Patients treated without or with trastuzumab showed comparable pCR rates (20.0% vs 20.3%; $P = 0.965$; Figure 2A). In Cox proportional hazards regression analyses, trastuzumab was not significantly associated with EFS in univariate analysis (hazard ratio, 0.97; 95% CI, 0.55–1.73; $P = 0.916$), and consistently showed no significant influence on EFS in multivariate analysis (hazard ratio, 0.79; 95% CI, 0.43–1.43; $P = 0.431$) (Table 3). Patients with trastuzumab usage had similar EFS compared with those without trastuzumab (log rank test, $P = 0.916$; Figure 3A).

Predictive Factors for pCR

The relationship of patient and tumor characteristics with pCR was analyzed in univariate and multivariate logistic regression analyses (Table 2). Univariate analysis suggested that patient's age along with menopausal status, clinical tumor size, clinical nodal status, tumor histology, Ki67 score, neoadjuvant chemotherapy, and trastuzumab usage showed no significant influence on the pCR rate. Negative HR status (OR, 3.72; 95% CI, 1.74–7.93; $P = 0.001$) and histological grade 3 (OR, 2.58; 95% CI, 1.22–5.44; $P = 0.013$) were significantly associated with higher pCR rates. Multivariate analysis confirmed that negative HR status (OR, 5.33; 95% CI, 2.11–13.45; $P < 0.001$) and histological grade 3 (OR, 3.25; 95% CI, 1.39–7.62; $P = 0.007$) were independent predictors for pCR.

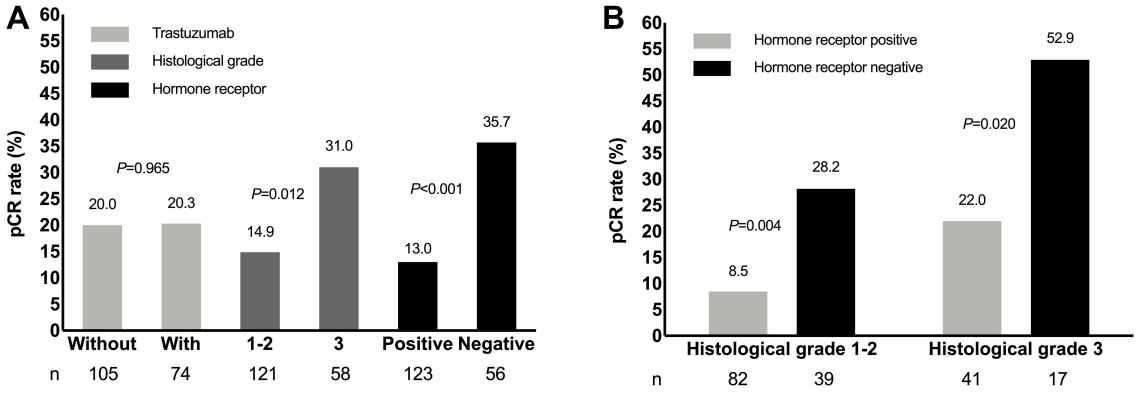

Figure 2 Pathological complete response rates according to treatment and tumor characteristics. (**A**) (light-gray box) pCR rates of tumors treated with trastuzumab versus without trastuzumab, (dark-gray box) pCR rates of tumors with histological grade 3 versus histological grade 1–2, (black box) pCR rates of tumors with negative HR status versus positive HR status; (**B**) pCR rates of tumors with negative HR status versus positive HR status in subsets of histological grade 3 and histological grade 1–2, respectively.

Abbreviations: HR, hormone receptor; pCR, pathological complete response.

Table 3 Associations of EFS with Patient and Tumor Characteristics in Univariate and Multivariate Cox Proportional Hazards Regression Analyses

Characteristics	Univariate Analysis			Multivariate Analysis		
	Hazard Ratio	95% CI	*P* value	Hazard Ratio	95% CI	*P* value
Age, years (≤50 vs >50)	0.72	0.40–1.31	0.287	0.66	0.26–1.67	0.380
Menopausal status (pre vs post)	0.87	0.48–1.57	0.632	1.10	0.43–2.84	0.841
Clinical tumor size (T1–2 vs T3–4)	0.70	0.35–1.37	0.294	0.66	0.28–1.52	0.328
Clinical nodal status (N0 vs N+)	0.69	0.39–1.23	0.205	0.45	0.17–1.22	0.117
Histology (ductal vs other)	0.87	0.12–6.39	0.891	1.23	0.16–9.74	0.842
Histological grade (3 vs 1–2)	1.00	0.55–1.83	0.992	1.15	0.60–2.19	0.670
Ki67 score (>35% vs ≤35%)	0.99	0.56–1.75	0.975	0.88	0.47–1.66	0.689
HR status (negative vs positive)	1.06	0.58–1.93	0.855	0.74	0.15–3.70	0.713
Trastuzumab (with vs without)	0.97	0.55–1.73	0.916	0.79	0.43–1.43	0.431
Pathological response (pCR vs non-pCR)	0.35	0.14–0.89	0.028	0.26	0.09–0.74	0.012
Neoadjuvant chemotherapy (combined vs sequential)	1.06	0.60–1.86	0.851	0.97	0.51–1.86	0.930
Adjuvant radiotherapy (yes vs no)	1.23	0.65–2.32	0.531	0.68	0.23-2.06	0.498
Adjuvant endocrine therapy (yes vs no)	0.89	0.49–1.60	0.692	0.45	0.09–2.18	0.318

Abbreviations: CI, confidence interval; EFS, event-free survival; HR, hormone receptor; pCR, pathological complete response.

Tumors with negative HR status had a significantly higher pCR rate in comparison with HR-positive disease (35.7% vs 13.0%; $P < 0.001$; Figure 2A). The pCR rate of histological grade 3 tumors was 31.0%, while tumors with histological grade 1–2 showed a pCR rate of 14.9% ($P = 0.012$; Figure 2A). We then employed HR status and histological grade as stratified factors for subgroup analysis. Results indicated that the entire population could be categorized into four subgroups showing extremely different pCR rates. Seventeen patients (9.5%) had tumors with negative HR status and histological grade 3, and demonstrated the highest pCR rate of 52.9% (Figure 2B). Eighty-two patients (45.8%) had tumors with positive HR status and histological grade 1–2, and showed the lowest pCR rate of 8.5% (Figure 2B).

Prognostic Factors for EFS

Univariate and multivariate Cox proportional hazards regression analyses were conducted to assess the prognostic value of patient and tumor characteristics on EFS (Table 3). In univariate analysis, patient's age along with menopausal status, clinical tumor size, clinical nodal status, tumor histology, histological grade, Ki67 score, HR status, trastuzumab administration, neoadjuvant chemotherapy, adjuvant radiotherapy, and adjuvant endocrine therapy were not significantly associated with EFS. However, achieving a pCR had a significant

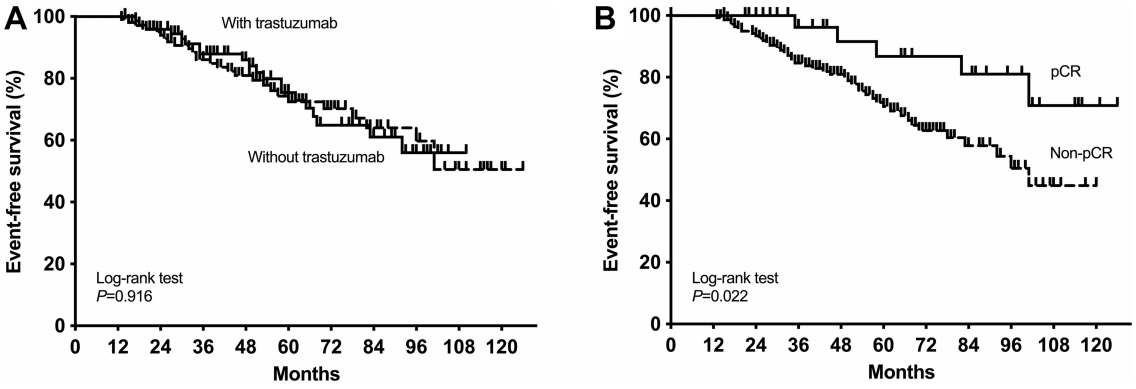

Figure 3 Event-free survival according to trastuzumab usage and status of pathological response. (**A**) EFS of patients treated with trastuzumab versus without trastuzumab; (**B**) EFS of patients with pCR versus without pCR.
Abbreviations: EFS, event-free survival; pCR, pathological complete response.

favorable impact on EFS (hazard ratio, 0.35; 95% CI, 0.14–-0.89; $P = 0.028$). In multivariate analysis, pCR was the only independent predictor for improved EFS (hazard ratio, 0.26; 95% CI, 0.09–0.74; $P = 0.012$). EFS was better among patients with pCR than among those with non-pCR (log rank test, $P = 0.022$; Figure 3B).

Discussion

The therapeutic efficacy of trastuzumab depends on the adaptive immunity of the immune system, and T lymphocytes function as critical effectors in the adaptive immune response.[27,28,40] Early investigations identified T lymphocytes as the main component of TILs in breast cancer.[41,42] For clinical utility, recent guidelines have standardized morphological evaluation of TILs based on H&E-stained slides.[30,37] In neoadjuvant research using this scoring methodology, a higher infiltrating degree of TILs has been identified and found to be associated with higher pCR rates and better survival in HER2-positive breast cancer.[35,43] Nevertheless, the therapeutic effect of trastuzumab in tumors with a low infiltrating level of TILs has not been reported. The present study was conducted to evaluate the therapeutic effect of trastuzumab on the pCR rate and EFS in neoadjuvant-treated HER2-positive disease with a low infiltrating level of TILs.

In the adjuvant FinHER trial, a significant interaction between TILs as a continuous parameter and trastuzumab therapy was reported, and higher infiltrating levels of TILs were found to be associated with increased trastuzumab benefit of survival.[44] Further analyses used a cut-off point of 50% to define a clinical subtype with a high immune infiltrate. Tumors with TILs ≥ 50% obtained a significant survival improvement with additional trastuzumab, while trastuzumab usage had no significant impact on survival for those with TILs < 50%.[44] On the contrary, in the adjuvant N9831 trial, patients with TILs ≥ 60% did not derive survival benefit from the addition of trastuzumab, whereas those with TILs < 60% showed significantly better survival with additional trastuzumab.[45] The associations between the infiltrating level of TILs and trastuzumab benefit remain controversial in HER2-positive breast cancer in the adjuvant setting.

In the neoadjuvant scenario, we found no evidence on the interaction between trastuzumab benefit and the infiltrating level of TILs. To our knowledge, our study is the first that investigated the therapeutic effect of trastuzumab on the pCR rate and EFS in neoadjuvant-treated HER2-positive tumors with a low infiltrating degree of TILs. The contradictory results of the FinHER and N9831 trials revealed the importance of a predefined cut-off point of TILs density in defining a clinical subtype with low immune infiltrate. Tumors with TILs ≥ 50% or 60% have been found to be infrequent and have excellent survival with fewer survival events in HER2-positive breast cancer.[32,35,43,45] The survival benefit of trastuzumab might not be significant in survival analyses for tumors with TILs ≥ 50% or 60%. On the other hand, tumors with TILs < 50% or 60% might have enough density of TILs for a trastuzumab effect, and, therefore, TILs < 50% or 60% might be unreasonable for representation of low immune infiltrate. Our study chose a relatively low cut-off point of 10%, and TILs < 10% was defined as a low level of immune infiltrate. Results indicated that the entire population had a pretty low pCR rate of 20.1%, and the pCR rate and EFS were comparable among patients treated with or without trastuzumab.

In predictive and prognostic analyses, trastuzumab administration was not associated with either the pCR rate or EFS. Among parameters in predicting pCR, the infiltrating degree of TILs has been widely accepted as a powerful indicator.[32–36,46] However, predictive factors for pCR in HER2-positive tumors with a low infiltrating level of TILs have been unknown. Our findings suggested a significant interaction of pCR with HR status and histological grade. Moreover, we identified a special clinical subtype with positive HR status and histological grade 1–2, which constituted 45.8% of our cohort with a pretty low pCR rate of 8.5%. Further research should focus on this common subtype in tumors with a low infiltrating level of TILs, which showed strong resistance to treatment. For prognostic factors, although the prognostic significance of pCR has been intensively studied,[10,12,26] this prognostic impact has not been validated in HER2-positive disease with a low infiltrating level of TILs. Our results indicated that only pCR independently correlated with favorable EFS in this special clinical subtype.

Our study has some limitations. For reasons of health insurance and medical cost, not all patients received trastuzumab in our cohort, and the present study was performed to study the therapeutic efficacy of trastuzumab. However, no patient in our study received pertuzumab, which had not been approved for NAT in our country before our research. Our findings should be carefully applied in HER2-positive breast cancer treated with dual HER2 blockade. Additionally, the small-sample, retrospective design of our study emphasizes the need for further prospective studies with larger populations of patients. Finally, currently, no validated cut-off point of TILs density has been determined in predictive or prognostic studies. The infiltrating level of TILs < 10% was used to define tumors with low immune infiltrate in our study.

Nevertheless, further research on the accurate cut-off point of TILs density in defining this special clinical subtype is warranted.

Conclusion

In neoadjuvant-treated HER2-positive breast cancer with a low infiltrating level of TILs (TILs < 10%), the pCR rate and EFS were comparable regardless of whether trastuzumab was administered or not. In this special subgroup, HR-negative status and histological grade 3 were independent predictors for pCR, and pCR was independently associated with the benefit of EFS.

Disclosure

The authors report no conflicts of interest in this work.

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