

Antigen-Presenting B-Cell Signature Predicts Response and Survival in HER2-Positive Breast Cancer

Po-Han Lin

National Taiwan University Hospital

Huang-Chun Lien

National Taiwan University Hospital

Wen-Hui Ku

Taipei Institute of Pathology

Chia-Jen Lin

Harvard Medical School

Bogang Wu

Mayo Clinic

Hui-Chen Wu

Mailman School of Public Health of Columbia University

Tzu-Chun Yen

National Taiwan University Hospital

Li-Wei Tsai

National Taiwan University Hospital

Chiao Lo

National Taiwan University Hospital

Ming-Yang Wang

National Taiwan University Hospital

Sung-Hsin Kuo

National Taiwan University

Chiun-Sheng Huang

huangcs@ntu.edu.tw

National Taiwan University Hospital

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Abstract

Neoadjuvant therapy (NAT) with HER2-targeted agents is the standard treatment for patients with HER2-positive early breast cancer (HER2 + EBC). Although high levels of tumor-infiltrating lymphocytes (TILs) are associated with higher pathologic complete response (pCR) rates and improved recurrence-free survival (RFS), the specific immune components responsible remain unclear. In this study, pretreatment tumor biopsies from 188 patients receiving HER2-targeted NAT were analyzed. Univariate analysis revealed that enrichment of TIL-B cells in tumors, high HER2 mRNA and low ESR1 mRNA expression were linked to pCR (both $p < 0.01$). Multivariate analysis confirmed TIL-B-cell enrichment as an independent predictor of pCR. Mature IgD/IgM-high B cells, but not immature RAG1 + B cells, independently predicted pCR. Transcriptomic profiling identified 265 upregulated genes in pCR tumors enriched in MHC class II antigen presentation pathways. Immunofluorescence demonstrated increased spatial interaction of mature B cells with CD4 + T cells in pCR tumors, consistent with active antigen presentation. The antigen presenting B-cell signature, integrated mature B-cell and MHC II profile, was the strongest independent predictor of pCR (OR = 3.008, 95% CI: 1.196–7.544, $p = 0.019$) and was also significantly associated with improved RFS (HR = 0.109, 95% CI: 0.014–0.872), highlighting B-cell-mediated antigen presentation as a clinically relevant biomarker in HER2 + EBC.

Introduction

Approximately 20% of breast cancer are classified as HER2-positive (HER2+), a subtype historically associated with higher recurrence rates and poorer prognosis¹. The introduction of HER2-directed agents has markedly improved survival outcomes compared with the pre-targeted therapy era². For most HER2 + early breast cancer (EBC), neoadjuvant therapy (NAT) with HER2-targeted agents and chemotherapy, has become the preferred treatment approach offering both high rates of pathologic complete response (pCR) and the opportunity for treatment de-escalation or escalation based on pathology at surgery^{3,4}. Achieving a pCR following NAT is widely recognized as a surrogate marker for favorable long-term outcomes in breast cancer^{5,6}.

Several factors may influence the likelihood of achieving pCR and better survival following NAT in HER2 + EBC. These include hormone receptor (HR) status, HER2 expression level, type of HER2-targeted regimen, and the tumor immune microenvironment^{7–9}. The tumor-infiltrating lymphocyte (TIL), a crucial component of the tumor microenvironment, has been extensively studied in recent years. In the NeoALTTO trial, baseline TILs were identified as a predictive biomarker for pCR, with higher TIL levels correlating with reduced recurrence rates¹⁰. Similarly, an analysis of the GeparQuattro and GeparQuinto trials, which involved trastuzumab-based NAT showed that every 10% increase in TILs was associated with a 12% increase in the probability of achieving pCR¹¹. Other studies have also reported that a high proportion of TILs is linked to improved survival outcomes^{12,13}.

Although TILs are recognized as predictive markers of pCR in HER2 + EBC receiving NAT, the composition and functional landscape of the associated immune microenvironment remain insufficiently characterized^{14–16}. A variety of immune cells, including neutrophils, monocytes, T cells, B cells, and plasma cells, can infiltrate tumors, but it remains unclear which subsets play the most decisive roles in predicting pCR. Furthermore, the functional characteristics of TILs associated with pCR in HER2 + EBC have not yet been fully elucidated. To address these gaps, we systematically profiled the immune landscape of HER2 + EBC to define the key immune cell subsets and mechanisms underlying TIL-mediated response. We hope these insights may facilitate the development of immune-based biomarkers and strategies to enhance treatment efficacy and patient survival.

Results

A total of 188 female patients with HER2 + EBC who received NAT were enrolled. Among them, 103 received trastuzumab-based NAT and 85 received dual HER2 blockades (trastuzumab plus pertuzumab). Clinical and pathological characteristics, including age, tumor size, lymph node status, ER status, and chemotherapy, did not differ significantly between the two groups (Supplementary Table 1).

Impact of clinical and tumor characteristics on pCR

The association between pCR and clinical, pathologic and gene expression of the tumors is illustrated in the supplementary table 1 and Fig. 1A. pCR was achieved in 55 patients. Clinical features such as age, tumor size, and nodal status showed no significant association with pCR. While dual-blockade therapy showed a trend toward higher pCR rates, this was not statistically significant (Supplementary table 1 and Fig. 1B).

HER2 mRNA expression was significantly higher in patients who achieved pCR ($p = 0.003$, Supplementary Fig. 1). Stratification by HER2 expression revealed that patients in the high-expression group (> 50 th percentile) had a significantly higher pCR rate with dual-blockade (46.2%) compared to trastuzumab alone (26.2%, $p = 0.047$, Supplementary Fig. 1B). No significant difference was observed in the low-expression group ($p = 0.587$). Patients with ER-negative tumors had a significantly higher pCR rate than those with ER-positive tumors (37.7% vs. 20%, $p = 0.008$), consistent with higher ESR1 mRNA expression in ER-positive tumors ($p < 0.001$), indicating an inverse relationship between ER status and pCR.

Mutations in TP53 (46.8%), PIK3CA (13.3%), and PTEN (22.9%) were identified by RNA sequencing but showed no significant association with pCR (Supplementary table 1).

B-Cell Enrichment Defines the Immune Landscape of pCR Tumors

RNA sequencing revealed 7,539 genes associated with pCR. Forty-one genes were upregulated ≥ 4 -fold in pCR tumors, of which 28 were immunoglobulin-related, including IGLV3-27, IGKV1-16, IGLC1, IGLC2,

IGLC3, IGLC4, IGHG1, IGKV1-5, IGLV1-44 and others (Fig. 2A). Gene Set Enrichment Analysis (GSEA) identified 3,669 significant immunologic gene sets among the C7 immune category (4586 gene sets). There were 401 and 343 gene sets involved in B and CD8 T cells, respectively (Fig. 2B).

We further deconvoluted the bulk RNA sequencing data using TIMER2.0 to estimate the relative abundances of major immune cell populations, including T cells, B cells, myeloid cells, and other immune subsets¹⁷. B-cell was the most dominant cell type among the TILs (Supplementary Fig. 2). We found that B-cell infiltration was significantly higher in pCR tumors (Fig. 2C). CD8 + T-cell and total T-cell counts showed an increasing trend but were not statistically significant (Figs. 2D and 2F). Other immune cell types were not associated with pCR.

To verify the reliability of RNA-based immune deconvolution, TIL density quantified on histopathology slides was correlated with RNA-derived immune cell estimates across 35 matched samples, demonstrating strong concordance between the two approaches ($R = 0.86$, $p < 0.001$, Fig. 2G). In patients with pCR, tumors were infiltrated by abundant immune cells, particularly CD19 + B cells, as shown by IHC (Fig. 2H). In contrast, tumors from non-pCR patients had low TIL and B-cell presence (Fig. 2I), well-consistent with RNA-based estimates.

B cell counts as the dominant factor for predicting pCR

Since the HER2 expression, ESR1 expression, type of targeted therapy, and TILs were all associated with pCR, we performed a multivariate analysis to identify the most influential factor. The logistic regression model revealed that a high B-cell count was the only predictor of pCR (hazard ratio [HR] = 2.307; 95% confidence interval [CI]: 1.110–4.796; Table 1).

Table 1
Univariate and multivariate logistic regression analysis of the pathologic, therapeutic and immunologic factors on pCR

	95% confidence interval			
	Odd ratio	Low	High	P value
Univariate				
Her2 expression (high vs. low)	2.195	1.149	4.193	0.016
ESR1 expression (high vs. low)	0.456	0.239	0.870	0.016
NAT dual blockade vs. trastuzumab	1.534	0.816	2.884	0.183
B cell counts (high vs. low)	2.658	1.375	5.139	0.003
CD8 T cell counts (high vs. low)	2.031	1.068	3.861	0.031
Multivariate				
Her2 expression (high vs. low)	1.630	0.794	3.345	0.183
ESR1 expression (high vs. low)	0.545	0.268	1.107	0.093
NAT dual blockade vs. trastuzumab	1.333	0.681	2.609	0.402
B cell counts (high vs. low)	2.307	1.110	4.796	0.025
CD8 T cell counts (high vs. low)	1.392	0.674	2.873	0.371

Maturity of B Cells, Not Quantity, Predicts Therapeutic Response

Patients with “B cell-high” had a better pCR rate than those with “B cell-low”. We defined tumors with deconvoluted B cell counts > 100 as “B cell-high” (n = 81). Among them, 35 patients (43.2%) achieved pCR—significantly higher than the pCR rate in “B cell-low” patients (18.7%, $p < 0.001$). However, among “B cell-high” patients, 46 cases (56.8%) did not achieve pCR, prompting further analysis to explore differences within this subgroup.

As shown in Figs. 3A and 3B, overall B cell counts were comparable between pCR and non-pCR cases. Expression of the pan-B cell marker CD19 was similar between groups (Fig. 3C). Markers, which represent mature B cells, including CD79a, IgM, IgD, and several immunoglobulin genes, were significantly upregulated in pCR tumors (Figs. 3D–3I). Conversely, RAG-1, associated with immature B cells, was higher in non-pCR tumors (Fig. 3E). These results suggest that mature B cells dominate in tumors achieving pCR.

Antigen Presentation as a Key Function of Tumor-Infiltrating B Cells

In the “B cell-high” subgroup, 265 genes were at least 2-fold upregulated in pCR cases. STRING network analysis of 256 of these genes revealed strong enrichment in MHC class II-mediated antigen presentation pathways (Fig. 4A and 4B). This enrichment suggests that mature B cells in pCR tumors may actively function as antigen-presenting cells (APCs). Such antigen-presenting activity might amplify local antitumor immunity and facilitate more effective tumor eradication during HER2-targeted therapy.

Major genes of MHC II antigen-presentation, which include CD74, CIITA, and HLA-DR/DQ ¹⁸, were significantly upregulated in pCR tumors across both the full cohort and “B cell-high” subgroup (Fig. 4C and 4D). During cognate B-T cell interaction, CD79a is involved in the MHC II presentation and mediated the activation and proliferation ¹⁹. CD79a was also elevated in pCR cases (Fig. 3D).

Spatial Coupling of B and CD4⁺ T Cells Indicates Active Antigen Presentation in pCR cases

To investigate whether mature B cells actively engage in antigen presentation within the tumor microenvironment, we performed multiplex immunofluorescence to assess the spatial relationship between mature B cells (IgM⁺/IgD⁺, green) and CD4⁺ T cells (red) (Fig. 5A). Frequent co-localization of these two cell types (appearing as yellow overlap) was observed in pCR tumors but was markedly reduced in non-pCR cases. Quantitative analysis confirmed that B–CD4⁺ T-cell interactions were significantly more abundant in tumors achieving pCR ($p < 0.001$; Fig. 5B). These spatial co-localizations suggest that mature B cells may function as local antigen-presenting cells, engaging CD4⁺ T cells through MHC class II–mediated signaling to potentiate antitumor immune activation in responders.

Remarkable Predictive and Prognostic Value of the Antigen Presenting B-cell Signature

Based on these findings, we developed a composite gene signature, named antigen presenting B-cell signature, by integration of the immunoglobulin gene expression, low RAG-1 levels, and key MHC II antigen presentation genes (Supplementary table 2).

Patients were divided into two groups: with high or low signature profile. Multivariate analysis identified the antigen presenting B-cell signature as the strongest independent predictor of pCR (OR = 3.008, 95% CI: 1.196–7.544, $p = 0.019$), after adjusting for ESR1, ERBB2, and TIL components (Table 2). This OR is higher than that of the B-cell count for predicting pCR (Table 2). We then analyzed the impact of this signature on recurrence-free survival (RFS). The multivariate Cox regression revealed that patients with a high signature score had significantly improved RFS (HR = 0.109, 95% CI: 0.014–0.872), independent of pCR status and other covariates (Table 2).

Table 2

Multivariate analysis of the clinicopathologic and immunologic factors on pCR and the recurrence-free survival.

pCR rate	95% confidence interval			
	Odd ratio	Low	High	P value
Her2 expression (high vs. low)	1.728	0.830	3.598	0.144
ESR1 expression (high vs. low)	0.540	0.263	1.110	0.094
NAT dual blockade vs. trastuzumab	1.324	0.668	2.625	0.421
B cell counts (high vs. low)	1.483	0.637	3.453	0.361
CD8 T cell counts (high vs. low)	1.082	0.496	2.359	0.843
Antigen presenting B-cell signature (high vs. low)	3.004	1.196	7.544	0.019
95% confidence interval				
Recurrence-free survival	Hazard ratio	Low	High	P value
Her2 expression (high vs. low)	2.451	0.812	7.391	0.112
ESR1 expression (high vs. low)	0.408	0.137	1.209	0.106
pCR status	0.518	0.165	1.633	0.262
B cell counts (high vs. low)	2.183	0.843	5.655	0.108
CD8 T cell counts (high vs. low)	1.581	0.618	4.046	0.339
Antigen presenting B-cell signature (high vs. low)	0.109	0.014	0.872	0.037

Discussion

We demonstrated that tumor infiltrated B cells, particularly mature B-cell with MHC II presentation subsets, are critical determinants of response to NAT in HER2 + EBC. While prior studies highlighted TILs broadly as predictors of pCR, our findings refine this understanding by identifying mature antigen presenting B cells as the dominant immune cell population driving high pCR rate as well as favorable survival.

NAT had become the standard of care for most HER2 + EBC and pCR had been considered as a surrogate marker positively correlation to the prognosis^{5,6}. Meta-analysis, clinical trials and the large-scale real-world studies revealed that pCR refer to superior recurrence-free and overall survival^{5,6,20,21}. When patients did not have a pCR, adjuvant trastuzumab emtansine or trastuzumab deruxtecan

significantly improved the survival of patients without pCR^{22,23}. However, some patients with residual tumor after NAT were treated with adjuvant trastuzumab emtansine or trastuzumab deruxtecan and some patients with pCR still recur. Thus, the strategies to increase pCR rates could still be important to improve the prognosis of HER2 + EBC, and a marker to predict not only pCR but also survival is also crucial.

Multiple factors affect the likelihood of achieving pCR in HER2-positive EBC, including hormone receptor status, HER2 expression levels, treatment regimens, and the tumor immune microenvironment^{6,11,12}. Taxane in combination with dual blockade therapy could achieve the highest pCR rate, compared to other regimens, and currently had become the standard regimen in the NAT setting⁹. The mechanism of the trastuzumab and pertuzumab attacking HER2-positive cancer cell is through the antibody-dependent cell-mediated cytotoxicity by nature killer cells and monocytes^{24,25}. Although innate immunity plays the frontline role to attack tumor cells, the adaptive immunity is the majority to fight against cancer^{26,27}. The CD8 + T cells are the effector to against tumor and the memory adaptive immunity, such as memory CD8 + T cell subsets, can mediate long-lived anti-tumor immunity²⁸, explaining the reason of the favorable RFS or overall survival^{26,27,29}. However, trastuzumab and pertuzumab do not directly activate CD8 + T cells. Classically, CD4 + Th1 cells play a crucial role in activating CD8 + T cells³⁰. CD4 + Th1 cells recognize antigen presented by the antigen presenting cells through MHC II molecules; then, the CD4 + Th1 cells activate CD8 + T cells by secreting cytokines, such as interferon-gamma, that enhance CD8 + T cell survival, memory formation, and cytotoxic function against tumors^{30,31}. In our study, the infiltrated B cells act as antigen presenting cells and significantly contact with CD4 T cells (Fig. 5). This interaction is a key part of the cell-mediated immune response, where Th1 cells act as orchestrators, boosting the cytotoxic functions of CD8 + T cells to fight again cancer. A recent mouse model that implanted with breast tumors expressing the rat-erbB2 oncoprotein highlighted the importance of antitumor immunity through the MHC-II mediated antigen presentation between B and CD4 T-cells³². This important mechanism translated into the clinical presentation: the antigen presenting B-cell signature illustrated the best performance for predicting pCR and RFS.

During B-cell development, immature B cells express RAG1/2 for V(D)J rearrangement, whereas mature B cells express IgD/IgM and constitute a major component of TIL and tertiary lymphoid structures (TLS)³³. Mature TIL-B and TLS-B cells are positively associated with favorable responses to cancer immunotherapy³⁴. Prior studies showed that tumor immune niches enriched with mature B cells contain higher proportions of CD4 + T cells, suggesting the importance of antigen presentation by B cells that enhances therapeutic responses in patients receiving immune checkpoint blockade and leading to the survival³⁴⁻³⁶.

Prior biomarker analyses from the CALGB 40601 and PAMELA trials demonstrated that B-cell and immunoglobulin G signatures outperformed TILs in predicting pCR and survival³⁷. HER2DX integrated immunoglobulin genes, HER2 gene, hormone receptor status, and clinical stage to generate risk score to predict survival of HER2 + EBC³⁸. As the risk score could not predict pCR well, another pCR likelihood

score was developed to predict pCR³⁸. Likewise, the pCR likelihood score does not predict survival well^{38,39,45}, which could be due to that high HER2 expression combined with low ESR1 expression predicts high pCR likelihood but not survival, whereas low expression of HER2 combined with high expression of ESR1 is associated with favorable RFS but not pCR rate^{10,44}. Thus, the HER2DX generated two independent scores to predict pCR and survival, separately. In contrast, our immune-focus signature predicted both pCR and survival in the same direction. Both HER2DX and our signature included immunoglobulin genes, suggesting the important role of the mature B cells on the prognosis of HER2 + EBC⁴². But a key distinction between our signature and HER2DX is that we include an antigen-presenting signature. Our mechanistic studies further confirmed that class II MHC-mediated antigen presentation is a critical function of TIL-B cells. Multivariate analysis revealed that the antigen-presenting B-cell signature exerted a greater influence on both pCR and DFS than hormone receptor status, HER2 expression levels, or treatment regimens. Therefore, our immune-focused, mechanistically grounded antigen-presenting B-cell signature alone is capable of predicting not only pCR but also RFS parallelly.

Our findings highlight a mechanistic link between B-cell functional maturity and anti-tumor immunity and suggest that assessing the antigen-presenting capacity of intratumoral B cells may refine patient stratification and guide personalized treatment strategies to improve clinical outcomes in HER2-positive EBC (Supplementary Fig. 3).

Methods

Patients

Female patients with HER2 + EBC who received NAT were enrolled in this study. All patients had histologically confirmed HER2-positive breast cancer, defined as either 3 + HER2 staining by immunohistochemistry or 2 + with amplification confirmed by fluorescence in situ hybridization. Clinical staging at initial diagnosis was determined by whole-body computed tomography, and patients with distant metastatic disease were excluded.

All patients received NAT consisting of chemotherapy combined with HER2 targeted therapy. The regimen included at least four cycles of taxane-based chemotherapy plus trastuzumab, or dual HER2 blockade with trastuzumab and pertuzumab. This study was approved by the institutional review board (IRB No. 201809075RSD).

Pathological response was assessed by dedicated breast pathologists. pCR was defined as the absence of invasive carcinoma in both the breast and axillary lymph nodes after surgery. Postoperatively, patients received adjuvant anti-HER2 therapy based on their pCR status, treatment accessibility, and patient preference. The total duration of anti-HER2 therapy was one year, in accordance with the National Comprehensive Cancer Network Breast Cancer Guidelines. The decision to administer adjuvant radiation therapy was based on surgical type and the number of lymph nodes involved. Additionally, adjuvant hormone therapy was provided to patients with hormone receptor-positive tumors.

RNA sequencing

RNA was extracted from FFPE breast biopsy specimens obtained at diagnosis and confirmed as invasive carcinoma. Sequencing libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit. Briefly, mRNA was isolated from total RNA, fragmented, and reverse-transcribed to cDNA. After adaptor ligation and PCR enrichment, libraries were purified, quality-controlled, and quantified. Qualified libraries were sequenced on an Illumina NovaSeq platform with 150-bp paired-end reads.

The generated fastq was aligned using STAR to the human genome reference GRCh38 to calculate the TPM (Transcripts Per Million) for each gene⁴⁰. Feature Counts were used to generate a matrix of raw gene counts from aligned sequencing reads. The differential gene expression (DGE) analysis in between two patient groups (such as pCR vs. non-pCR) was carried by DESeq2⁴¹. Pathway enrichment analysis of DEGs was conducted using Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA)⁴². The STRING database (<https://string-db.org>) was used to construct and analyze protein–protein interaction networks selected genes⁴³.

Immunofluorescence study

Sections (4 µm) were prepared from FFPE breast biopsy specimens, deparaffinized, subjected to antigen retrieval, and blocked for nonspecific binding. Slides were incubated with primary antibodies against IgM (GeneTex, Irvine, USA), IgD (GeneTex, Irvine, USA), or CD4 (Roche Diagnostics, Basel, Switzerland). Bound antibodies were detected using poly-HRP–conjugated secondary antibodies, and nuclei were counterstained with DAPI. Stained sections were mounted on chambered coverslips and imaged using an FV3000 confocal laser scanning microscope (Olympus, Tokyo, Japan).

Statistics

The chi-square test and Fisher's exact test were used to assess the significance of the variance between each group. Kaplan-Meier analysis was used to estimate recurrence-free survival (RFS). Odds ratio (OR) of pCR was estimated by logistic regression model. Cox proportional hazards regression analysis was conducted to determine the hazard ratios (HRs) of RFS with corresponding 95% confidence intervals (CI) for various factors. All *p* values were two-sided, and those less than 0.05 were regarded as statistically significant.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

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Author contributions:

Po-Han Lin and Chiun-Sheng Huang designed the study. Huang-Chun Lien and Wen-Hui Ku reviewed the pathology and calculated the tumor infiltration lymphocytes. Hui-Chen Wu, Tzu-Chun Yen, Li-Wei Tsai, Chiao Lo, Ming-Yang Wang and Sung-Hsin Kuo analyzed the clinical data, performed experiments and bioinformatics analysis. Chia-Jen Lin and Bogang Wu gave critical reviews on this study and manuscript. The authors have read and approved of the final manuscript.

Competing interests

Po-Han Lin reported receiving grants to institutions from AstraZeneca and Pfizer Inc. and received speaker honoraria from AstraZeneca, Pfizer, and Takeda outside the submitted work. Chiun-Sheng Huang reports receiving research grants from Aston Sci, Astra Zeneca, Daiichi Sankyo, EirGenix, Eli Lilly, Gilead, MSD, Novartis, OBI Pharma, Pfizer, Roche, Seagen; receiving honoraria from AstraZeneca, Daiichi Sankyo, Eli Lilly, Novartis, Pfizer, Roche; and advisory fees from AstraZeneca, Daiichi Sankyo, Eli Lilly, Novartis, Pfizer, Roche, outside the submitted work. No other disclosures have been reported.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of the National Taiwan University Hospital (201809075RSD), and informed consent was obtained from all the participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Figures

Figure 1

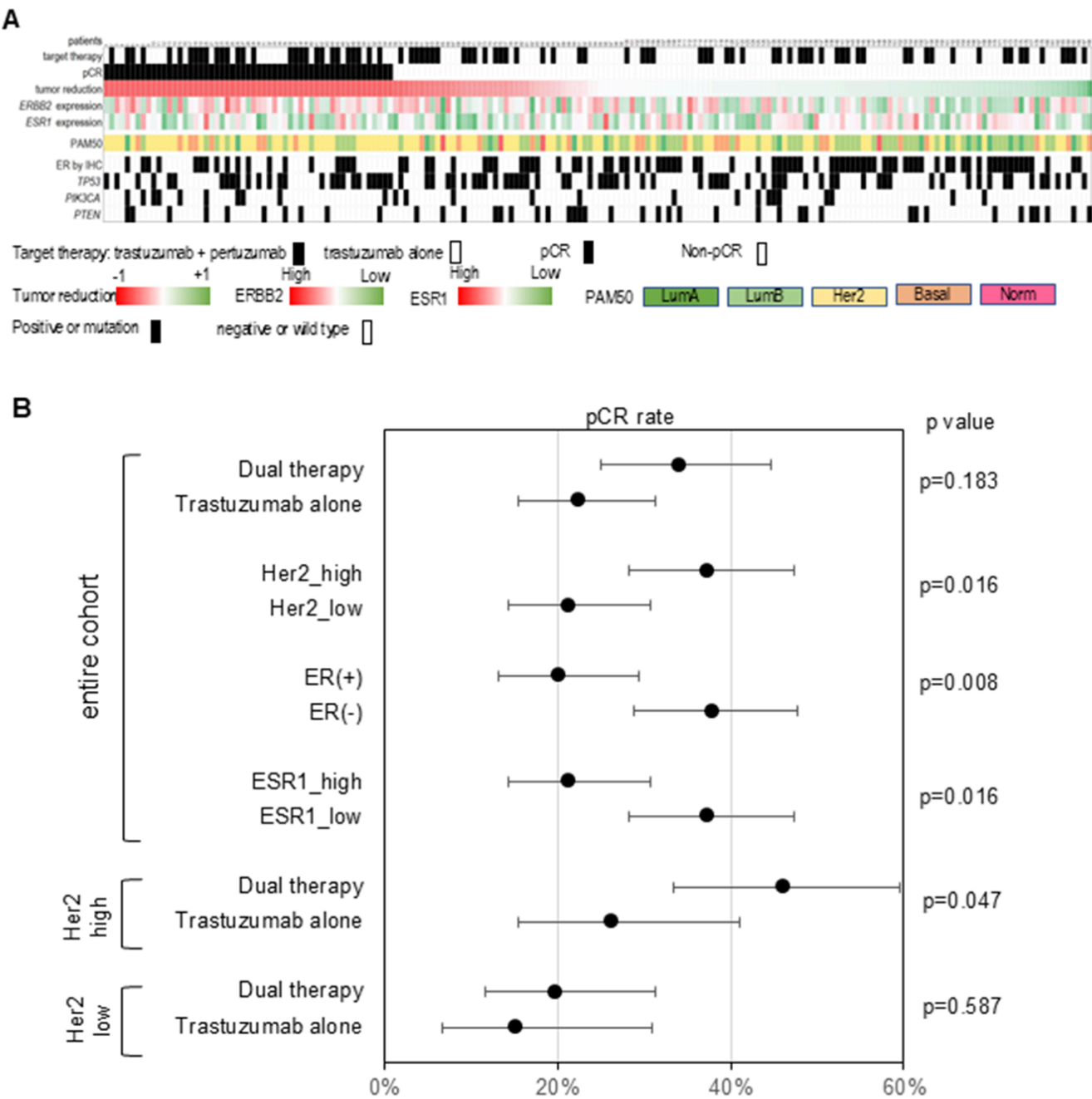


Figure 1

Association between tumor characteristics and pCR

(A) Genomic landscape of the enrolled patients. Each column represented one patient. (B) Comparison of the pCR rate for the patients treated with NAT in the different conditions. Entire cohort: overall 188 patients; HER2_high: patients with high-expression of HER2 (>50th percentile, N=94); HER2_low: patients

with low-expression of HER2 (<50th percentile, N=94); ER(+): positive expression of ER by IHC staining; ER(-): negative expression of ER by IHC staining; ESR1_high: patients with high-expression of ESR1 (>50th percentile, N=94); ESR1_low: patients with low-expression of ESR1 (<50th percentile, N=94).

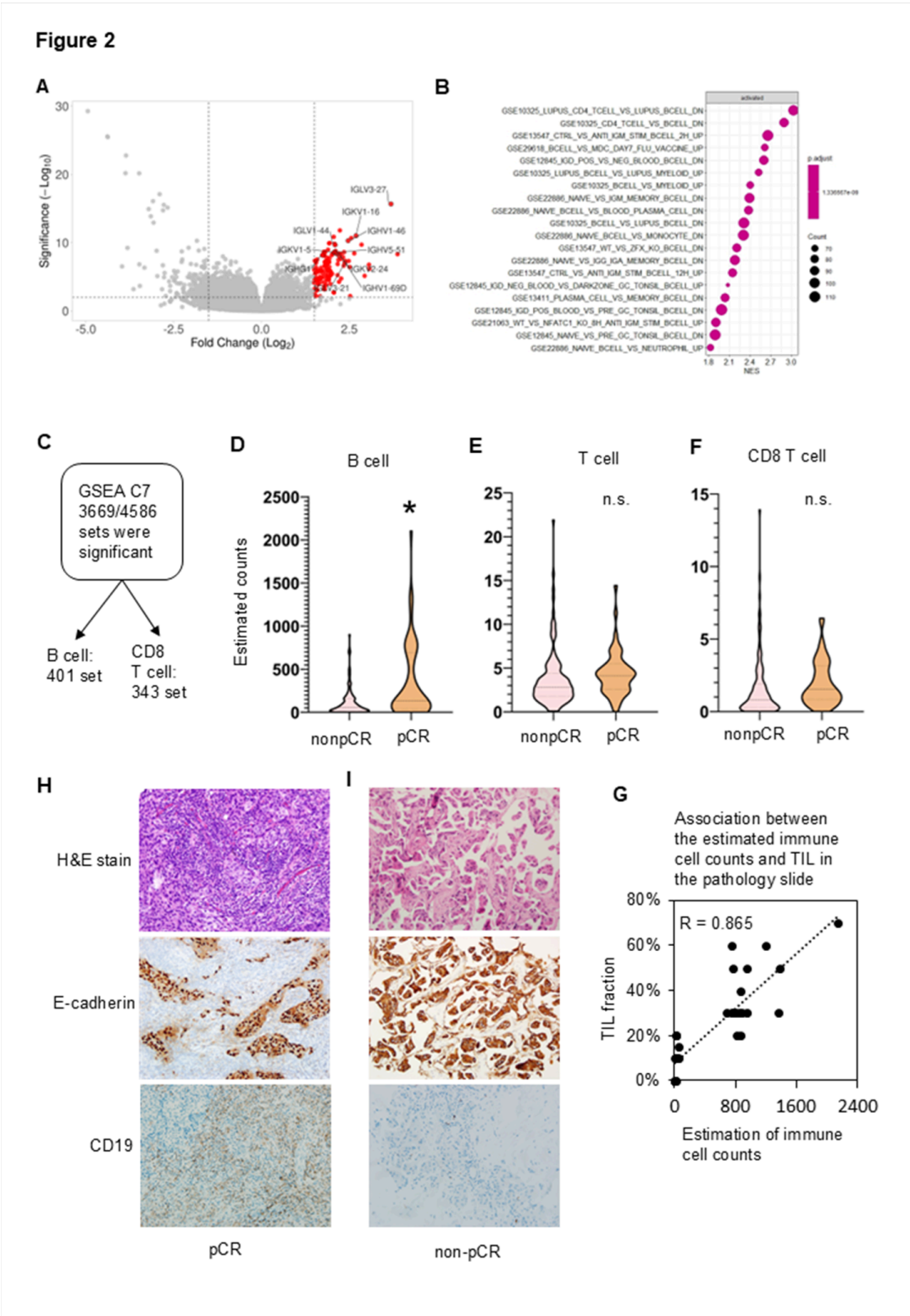


Figure 2

The immune microenvironment.

(A) A volcano plot illustrated differential expression of the entire cohort with and without pCR (generated by Clustvis). The red dots represented genes being 4x higher expression in pCR patients. (B) Bubble plot depicting the pathway enrichment analysis. Pathways activated (purple) are shown, with the x-axis representing the normalized enrichment score (NES). (C) Of the 4586 immune pathways, there were 3369 enrichment pathways achieving significant. A total of 401 gene sets were involved in B-cell, while 343 gene sets were involved in CD8 T-cell. The violin plots showed the distributions of the (D) B-cell counts, (E) T-cell counts and (F) CD8 T cell counts. (H) Immunohistochemical staining of H&E, E-cadherin and CD19 in the pCR and non-pCR related tumors. E-cadherin represented cancer cells while CD19 stands for B-cells. (I) Linear regression revealed the positive correlation of the TILs deconvoluted from RNA sequencing and counted by a pathologist ($p < 0.001$). * Indicated significance of p value.

Figure 3

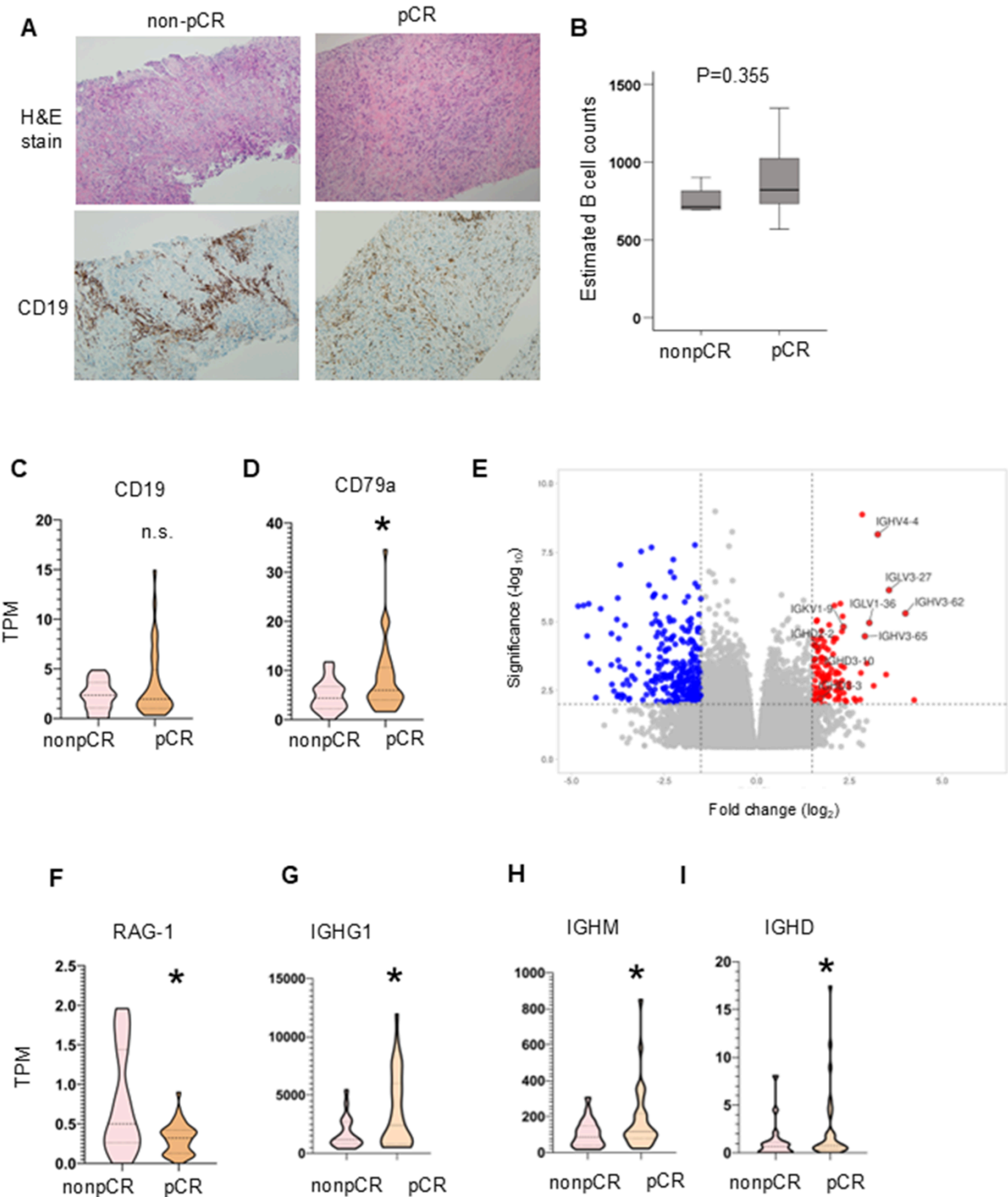


Figure 3

Subgroup analysis of patients with the characteristics of “B Cell-High”.

(A) Immunohistochemical staining of the H&E and CD19 in the pCR and non-pCR related tumors. (B) The estimated B-cell counts from the deconvolution of the RNA sequencing in the pCR and non-pCR tumors. (C)(D)(F-I) Target gene expression in the pCR and non-pCR tumors. (E) A volcano plot illustrated

differential expression of the patients with and without pCR. The red dots represented genes being 4x higher expression in pCR patients. * Indicated significance of p value.

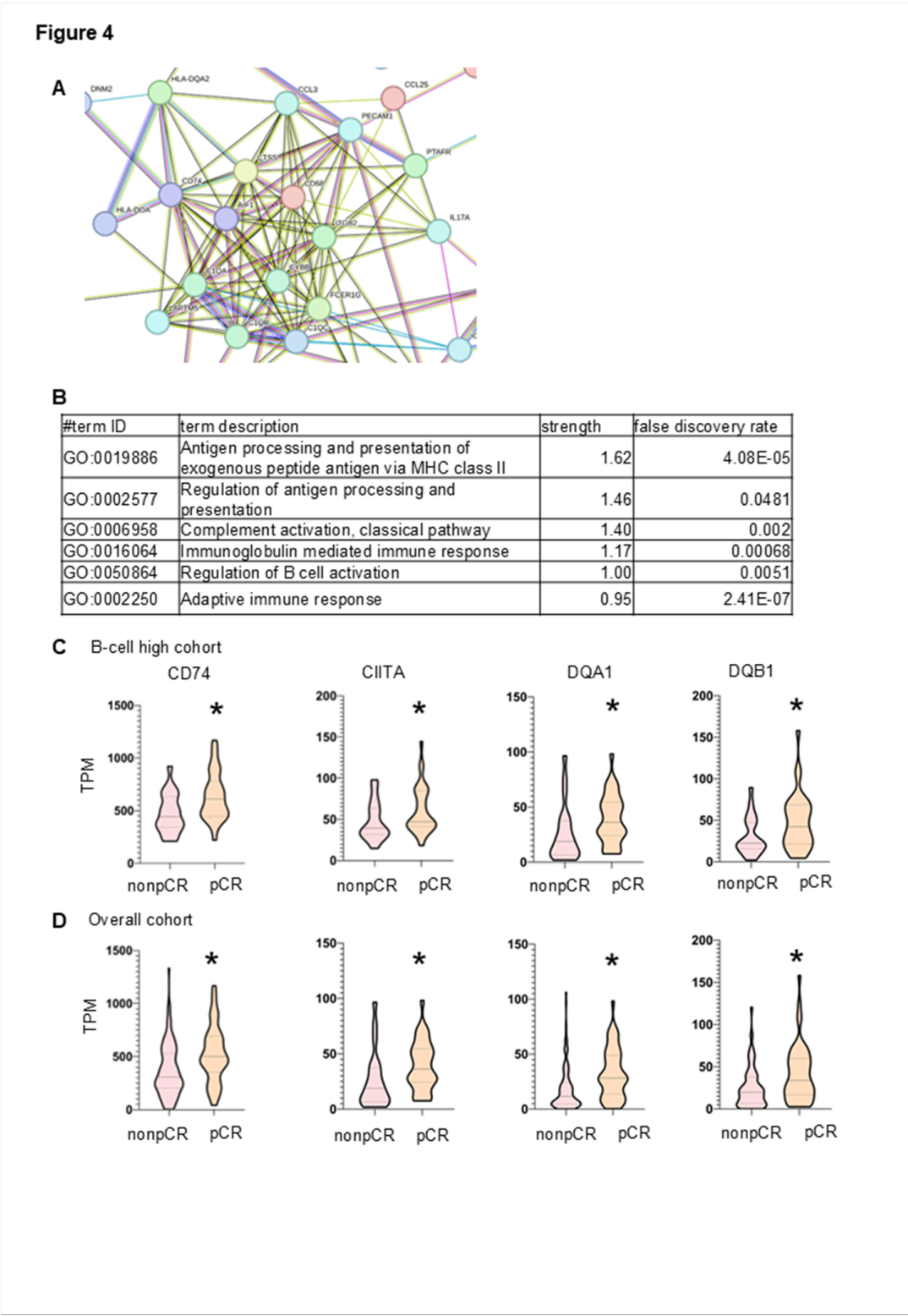


Figure 4

MHC II mediated antigen presentation.

(A) STRING analysis of proteins significantly expressed in the pCR tumors. (B) significant pathways in the gene ontology analysis. (C) Target gene expression in the pCR and non-pCR tumors in the subgroup patients with “B Cell-High”. (D) Target gene expression in the pCR and non-pCR tumors in the entire cohort. * Indicated significance of p value.

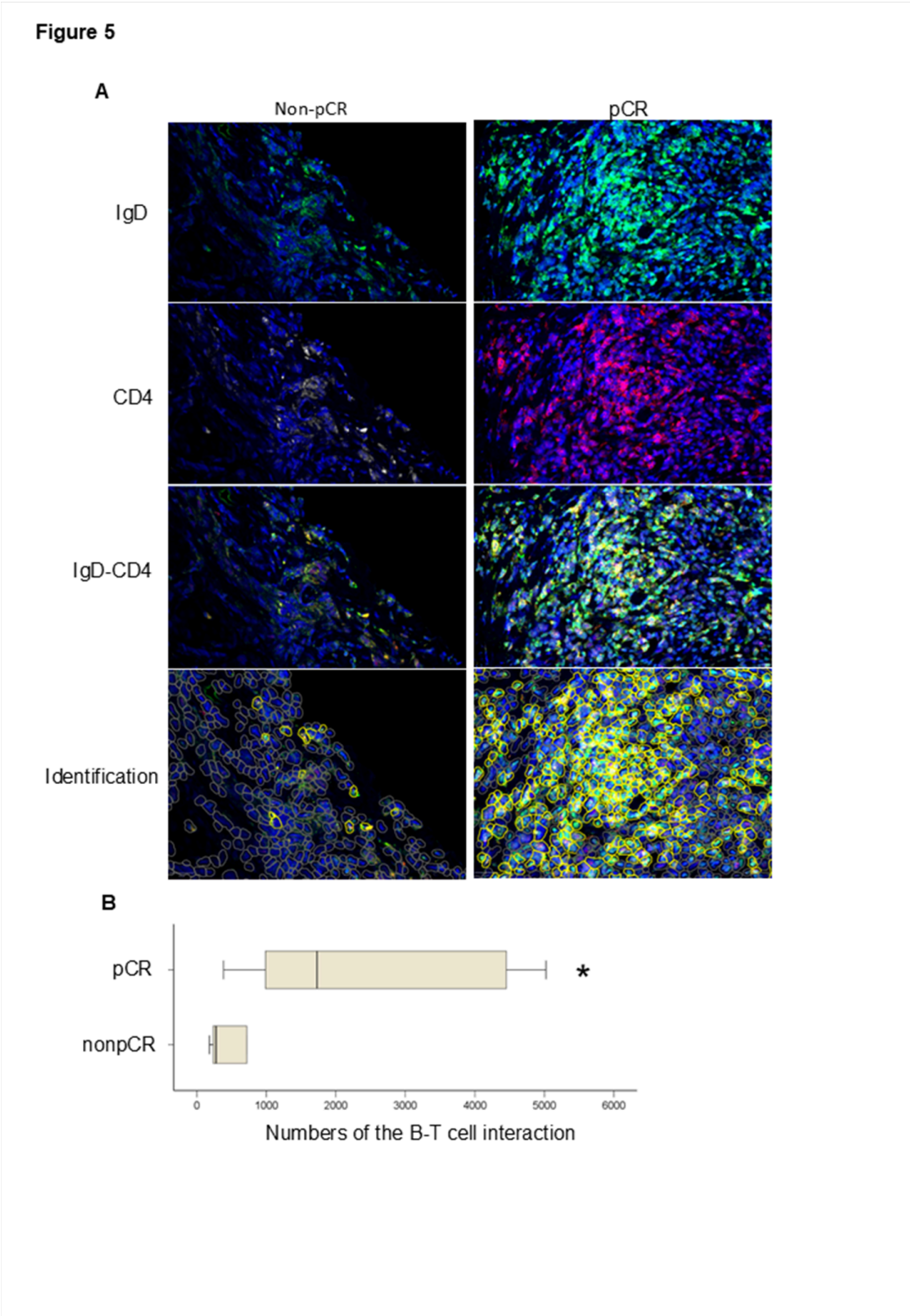


Figure 5

Spatial relationship between B and T cells in the tumor microenvironment.

(A) Immunofluorescence staining of the IgD (green) and CD4 (red). The B-T cell interactions were presented as yellow color and can be circled in the identification photos. (B) barogram represented the number of the B-T cells interaction in the pCR and non-pCR tumors. * Indicated significance of p value.

Supplementary Files

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