INNOVATIONS JOURNALS

Brief Report

Prognostic Impact of Cytotoxic CD4 T Cells in Tumor Immune Microenvironment of Patients with Breast Cancer

Kyrillus S. Shohdy,[1,2] Doaa S. Almeldin,[1,3] Ramy Ghaly,[1] Loay Kassem,[1] Olivia Pagani[4]

[1]Department of Clinical Oncology, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt
[2]Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY, USA
[3]Department of Radiation Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA
[4]Interdisciplinary Cancer Service, Hopital Riviera-Chablais, Rennaz, Geneva University Hospitals, Switzerland

Address correspondence to: Kyrillus S. Shohdy (shohdyks@cu.edu.eg).

Source of Support: None. Conflict of Interest: None.

Received: June 17, 2021; Revision Received: Aug 23, 2021; Accepted: Sep 14, 2021

Shohdy KS, Almeldin DS, Ghaly R, Kassem L, Pagani O. Prognostic impact of cytotoxic CD4 T cells in tumor immune microenvironment of patients with breast cancer. *J Immunother Precis Oncol*. 2022; 5:7–9. DOI: 10.36401/JIPO-21-15.

Tumor-infiltrating lymphocytes (TILs), as well as immune gene signatures, have shown prognostic and predictive potential in triple-negative and human epidermal receptor-2 (HER2) positive breast cancer subtypes.[1,2] The primary immune response was attributed to the cytotoxic activity of CD8+ T-cell infiltrate. The ratio between active cytotoxic CD8+ lymphocytes and inhibitory CD4+ regulatory T lymphocytes (Tregs) is believed to maintain homeostasis by immunologic surveillance and immune tolerance. The critical role of heterogeneous subsets of TILs beyond CD8+ cytotoxic predominance and regulatory negative signals in breast cancer remains insufficiently examined.

Cytotoxic CD4 T cells (CD4 CTLs) are a subset of CD4 T cells found to infiltrate the tumor immune microenvironment (TIME) and have cytotoxic activity in serval malignancies.[3–6] Recently, Oh et al[3] developed a gene signature defining the CD4 CTLs using single-cell RNA and paired T-cell receptor sequencing. The newly characterized CD4 CTLs maintain tumor cell cytotoxic activity through a major histocompatibility complex (MHC) class II-dependent fashion. However, the presence and prognostic value of CD4 CTLs in breast cancer TIME are unknown.

We tested a gene signature that identified the CD4 CTL subset within the T-cell basin in the breast cancer TIME and examined its association with breast cancer patients' outcomes in two independent breast cancer cohorts.

First, we extracted the transcriptomic and clinical outcomes of patients with breast cancer from the cancer genomic atlas (TCGA-BRCA) using the Genomic Data Commons.[7] Being a secondary analysis of publicly available data, institution review board approval and the need for consent were waived. Transcriptomic data were from treatment-naïve primary invasive breast cancer samples. Z-scores of the expression of the five genes defining active CD4 CTL (*ABCB1*, *APBA2*, *SLAMF7*, *GPR18*, and *PEG10*) were used to calculate the signature score using the principal component analysis (Fig. 1A). The final formula for the signature for each sample = [*ABCB1* × 0.46 + *APBA2* × 0.24 + *SLAMF7* × 0.59 + *GPR18* × 0.61 +*PEG10* × 0.11]. The CD4 CTL signature score was dichotomized into high vs. low scores using the median value (0.02). The abundance of other T-cell subpopulations was estimated using the CIBERSORT computational tool.[8] Overall survival (OS) and disease-free survival (DFS) were used as the primary prognosis endpoints and were estimated by the Kaplan-Meier survival curve.

Transcriptomic and clinical data of 1083 breast cancer patients were retrieved from TCGA-BRCA. The median score of the CD4 CTL-defining gene signature was 0.02 (range −4.45 to 4.79) (Fig. 1A). High signature scores were significantly more frequent in younger patients (< 55 years) (57% vs 42%, $p = 0.001$), nonluminal subtypes (65% vs. 45%, $p = 0.001$), and invasive duct carcinoma histology (76% vs 24%, $p = 0.008$).

In multivariate regression analysis, adjusting for age, tumor and node (TNM) stage, and subtype, higher CD4 CTL signature score was significantly associated with better DFS and OS hazard ratio (HR): 0.62, 95% CI: 0.42–0.96, $p = 0.03$ and HR: 0.66, 95%CI: 0.43–0.99, $p = 0.001$), respectively (Fig. 1B, 1C). In a stratified log-rank survival test, a higher score was associated with better OS among advanced T stage (T3–T4) ($p = 0.014$) and node-positive subgroups ($p = 0.016$).

We ran a correlation analysis between CD4 CTL with different immune cell infiltrate fractions using the

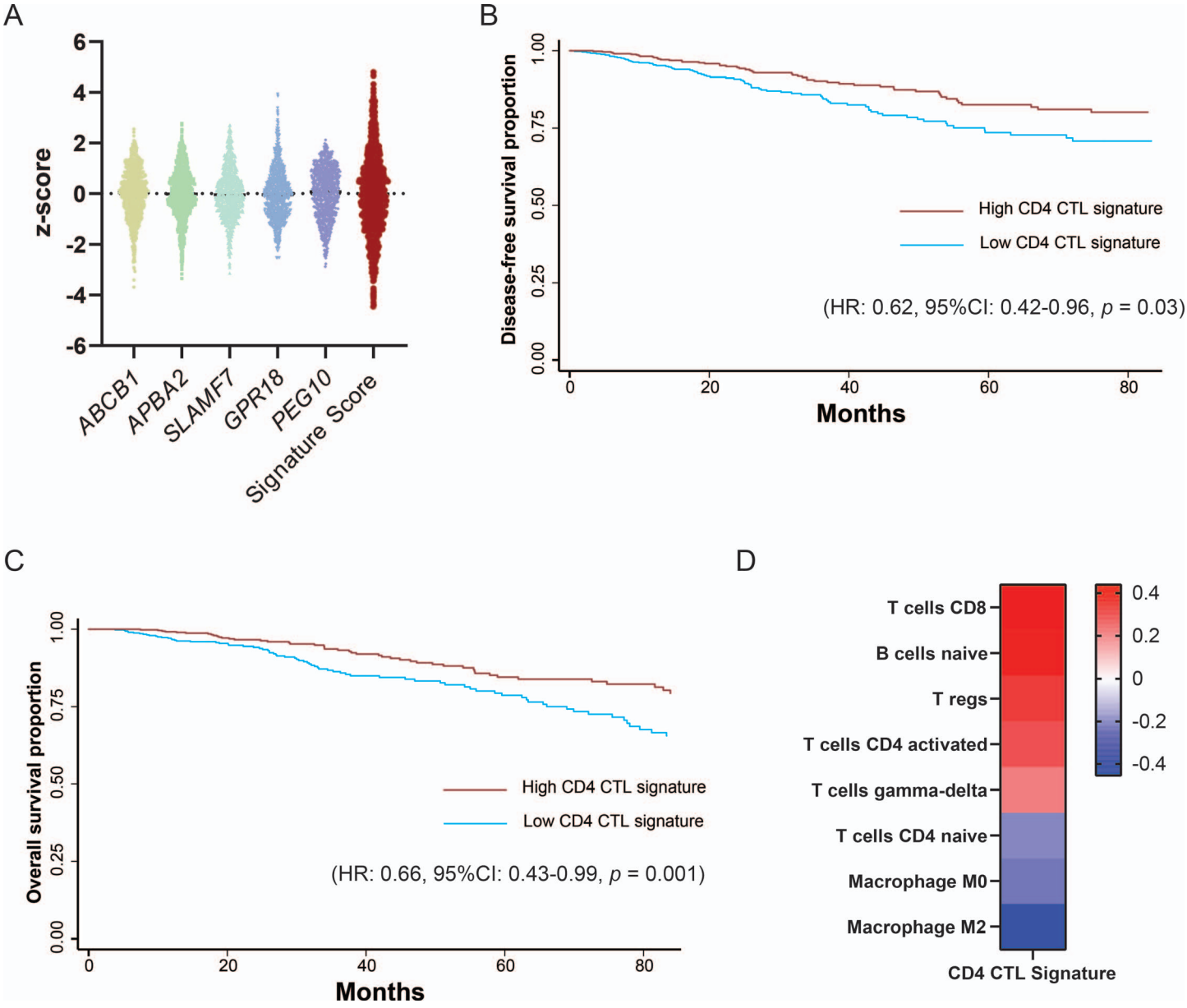

Figure 1. (**A**) The z-scores of the genes defining the cytotoxic CD4 T cell (CD4 CTL) signature across the TCGA-BRCA cohort. (**B**, **C**) Patients with high CD4 CTL were associated with significantly longer disease free-survival and overall survival. (**D**) Heatmap of r^2 of the correlation of CD4 CTL signature with key immune cells. HR: hazard ratio; T regs: T-regulatory cells.

Spearman correlation test. The analysis showed a significant positive correlation with CD8 T cells ($\rho = 0.44$, $p = 0.001$) and a significant negative correlation with macrophage M2 cells ($\rho = -0.45$, $p < 0.001$) (Fig. 1D). These data suggest that CD4 CTL is coenriched with favorable immune cells.

To validate our findings in a metastatic setting, we tested the CD4 CTL signature, using previously mentioned methods, on RNA sequence data derived from 146 patients with metastatic breast cancer from the Metastatic Breast Cancer Project.[9] High signature scores were significantly more frequent in younger patients (< 55 years) (55% vs 18%, $p = 0.007$). Moreover, high signature scores showed a trend toward significance with invasive duct carcinoma histology (56% vs 41%, $p = 0.08$). On the other hand, low signature scores were significantly more frequent in patients with homologous recombination deficiency (100% vs 46%, $p = 0.023$) and inflammatory breast cancer (86% vs 47%, $p = 0.046$). No significant correlation was observed between the signature score and tumor mutational burden in all cohorts ($\rho = -0.06$, $p = 0.50$) or in the triple negative subtypes ($\rho = 0.13$, $p = 0.56$). The site of the sample did not impact the signature score ($p = 0.57$). High signature scores were associated with numerically longer median distant metastasis-free survival (30 months [95% CI: 20–71] vs 21 months [95% CI: 5–47], $p = 0.2$).

Our analysis identified the CD4 CTL in the breast cancer TIME and showed a significantly good prognostic value indicating that CD4 CTL plays a critical role in the TIME of breast cancer, especially in young patients with nonluminal subtypes. CD4+ T cells with cytotoxic activity (CD4 CTL) have been observed in various immune responses.[3–6] These cells are characterized by their ability to secrete granzyme B and perforin and kill the target cells in an MHC class II-restricted fashion.[3] Although CD4 CTLs were once thought to be an in vitro artifact associated with long-term culturing, they have since been identified in vivo and shown to play critical roles in antiviral and antitumor immunity.[3–6] The presence of a CD4+ T cell subset that directly promotes cell-mediated immunity through other effector mechanisms remains unclear.

In a recent analysis on bladder carcinoma samples, single-cell RNA sequencing was done for more than 16,000 CD4+ T cells infiltrating tumor and nonmalignant tissue.[3] Several states of cytotoxic CD4+ T cells expressing cytolytic effector proteins were identified, some of which are enriched in tumors. CD4 CTL were clonally expanded in tumors and could kill autologous tumors ex vivo. CD4 CTL existed in discrete proliferating and nonproliferating states in tumors. A gene signature of cytotoxic CD4+ T cells was predictive of response to programmed cell death protein 1 (PD-1) blockade in an orthogonal RNA-seq dataset of metastatic bladder cancer patients treated with anti–PD-L1.[3] Overall, these findings highlight the importance of CD4+ T-cell heterogeneity and the relative balance between activation of cytotoxic CD4+ effectors and inhibitory regulatory cells for killing autologous tumors. Other immune cell infiltrate markers, such as granzyme B, *NKG2A*, and *NKG2D*, were not evaluated in this analysis to avoid potential overlapping expression from other T-cell subtypes. The present data set does not allow for detailed subgroup analysis according to treatment received, in particular for patients receiving immune checkpoint inhibitors.

In conclusion, the cytotoxic CD4 T cell is an emerging prognostic biomarker within the breast cancer immune microenvironment. Further dissection of CD4 CTL activity could carry a predictive signal for immunotherapy in patients with breast cancer. The CD4 CTL gene signature requires further validation in prospective trials as a predictor of response to immune checkpoint blockade.

References

1. Denkert C, Von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J Clin Oncol*. 2015;33:983–991.
2. Wang S, Zhang Q, Yu C, Cao Y, Zuo Y, Yang L. Immune cell infiltration-based signature for prognosis and immunogenomic analysis in breast cancer. *Brief Bioinform*. 2021;22:2020–2031.
3. Oh DY, Kwek SS, Raju SS, et al. Intratumoral CD4+ T cells mediate anti-tumor cytotoxicity in human bladder cancer. *Cell*. 2020;181:1612–1625.e13.
4. Takeuchi A, Saito T. CD4 CTL, a cytotoxic subset of CD4+ T cells, their differentiation and function. *Front Immunol*. 2017;8.
5. Thiault N, Husain M, Chen A, Larange A, Cheroutre H. CD4 Cytotoxic T cells play a critical role in anti-tumor protection. *J Immunol*. 2020;204(1 Suppl):241.20.
6. Hashimoto K, Kouno T, Ikawa T, et al. Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. *Proc Natl Acad Sci U S A*. 2019;116:24242–24251.
7. Genomic Data Commons Data Portal. National Cancer Institute. 2021. https://portal.gdc.cancer.gov/. Accessed May 4, 2021.
8. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12:453–457.
9. The metastatic breast cancer project. mbcproject.org/. Accessed May 4, 2021.