Original Article

Intratumoral CD8$^+$ T cells as a potential positive predictor of chemoimmunotherapy response in PD-L1-negative advanced gastric cancer patients: a retrospective cohort study

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Background: Previous studies have shown that PD-L1-positive advanced gastric cancer (GC) patients could achieve clinical benefit after receiving immune checkpoint inhibitors (ICI) in initial or subsequent therapy. A number of prospective studies such as Keynote-158 have demonstrated that PD-L1-negative patients who tested as microsatellite instability-high (MSI-H) or tumor mutational burden-high (TMB-H) can benefit from ICIs. In the search for more biomarker for immunotherapy, some studies showed that patients with a specific characteristic to tumor microenvironment (TME) were associated with better prognosis. This study aimed to explore the association between the TME and immunotherapy in PD-L1 negative GC patients.

Methods: This study was a retrospective cohort study. Twenty-six CPS PD-L1 negative stage IV advanced GC patients treated with chemoimmunotherapy in Shenzhen Hospital of Peking University were retrospectively enrolled according to the inclusion criteria. Their clinical characteristics were assessed and recorded by independent clinicians. Follow-up data was conducted through the Internet or visit. Respond to treatment was evaluated by RECIST 1.1. The primary outcome was progression-free survival (PFS). The level of tumor-infiltrating lymphocytes (TILs) was measured by multiplex immunofluorescence (mIF) among these patients. Cox proportional hazards analysis was performed to analyzed the correlation between PFS and clinical characteristics including TILs.

Results: Among 26 patients, 5 patients (19.2%) were on complete response (CR) and 9 patients (34.6%) were in partial response (PR), while 7 patients (26.9%) experienced stable disease (SD). Intratumoral CD8$^+$ T cells were obviously increased in CPS PD-L1 negative patients who responded to chemoimmunotherapy, compared with patients who did not respond (P=0.011). And higher level of CD8$^+$ TILs was demonstrated to associate with better PFS in CPS PD-L1-negative patients treated with chemoimmunotherapy (HR =23.70, 95% CI: 1.15–488.30, P=0.04).

Conclusions: Intratumoral CD8$^+$ TILs may be a potential positive predictive factor of clinical response for chemoimmunotherapy in PD-L1-negative advanced GC. However, the results need to be further confirmed in a cohort with more subjects due to a limited sample sizes in present study.

Keywords: Gastric cancer; PD-L1; chemoimmunotherapy; immune microenvironment; $CD8^+$ T cells

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Introduction

Gastric cancer (GC) ranks third among the most common causes of cancer-related death. At least 1 million people worldwide are diagnosed with gastric cancer each year, and the majority of patients are diagnosed at locally advanced or metastatic stage, with a low median survival rate (1). For decades, HER2-negative GC patients had limited treatment options at systemic therapy. So far in Asia, the first-line therapy for HER2-negative advanced GC or metastatic GC had recommended to either S-1 or capecitabine plus platinum, with a median survival of less than 12 months (2). For the past few years, immune checkpoint inhibitors (ICIs), represented by biomacromolecules that target programmed cell death protein-1 (PD-1) or PD-1 ligand-1 (PD-L1), have shown their therapeutic effect on GC.

Checkmate 649 trial demonstrates that patients with advanced GC of PD-L1 combined positive score (CPS) ≥5 can benefit from ICIs plus chemotherapy as the first-line therapy (3). Several clinical studies have also found that ICIs are generally preferred over traditional chemotherapy for PD-L1 positive patients of gastric cancer due to their excellent efficacy (4,5). Meanwhile, a meta-analysis showed that PD-L1-negative patients actually cannot benefit from ICIs, except for those tested as microsatellite instability-high (MSI-H) or tumor mutational burden-high (TMB-H) (5). Keynote-158 has showed that patients with MSI-H or TMB-H could also benefited from ICIs and these regimens have been approved by Food and Drug Administration (FDA). Moreover, several studies have shown that PI3KCA mutation, co-mutation in DNA damage response pathways or Epstein-Barr virus (EBV)-positive gastric cancer patients are likely to benefit from immunotherapy, and the presence of these biomarkers may indicate the presence of more neoantigens whose underlying mechanism was totally different from the role of PD-L1, and these suggested that in addition to PD-L1, patients with gastric cancer who benefit from ICI therapy may have other underlying mechanisms (6-8). Therefore, more biomarkers used to predict the immune response in PD-L1-negative patients with gastric cancer are needed, in order to expand the population that can benefit from immunotherapy.

Several studies have highlighted the importance of the tumor microenvironment (TME) in the development, metastasis, and migration of tumors (9,10). In a real-world immunotherapy cohort of patients with non-small cell lung cancer, immunotherapy with high tumor-infiltrating lymphocytes (TILs) not only showed better objective response rate (ORR), but also significantly improved disease control rate (DCR) and overall survival (OS) compared with chemotherapy, suggesting that TILs may be a predictive biomarker of the efficacy of immunotherapy (11). In addition, malignant melanoma patients harboring BRAF V600E/K-mutation with higher level of TILs was found to have an improved prognosis and better response to immunotherapy (12). However, the predictive value of immune cells in advanced GC is still unknown. In this study, we aimed to explore the function of multiple immune cells in immunotherapy. The levels of immune cells in the TME of each patient determined by mIF were used to explore the correlation between the TME and the efficacy of chemoimmunotherapy for PD-L1-negative advanced gastric cancer patients. We present the following article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-644/rc).

Methods

Patient and study design

This retrospective cohort study was designed to assess the association between the response to chemoimmunotherapy and the characteristic of immune microenvironment. We analyzed data from advanced GC patients treated with ICIs-based regimens who enrolled from July 2019 to January 2021 at the department of oncology, Peking University Shenzhen Hospital. Signed written informed consent forms were obtained from patients prior to first dose of therapeutic regimen. Treatment followed the institutional guidelines was performed. Clinicians collected demographic and clinical pathology characteristics from the patient's electronic medical records, including staging, metastasis, age, surgical margin status and so on. The inclusion criteria

were as follows: (I) patients with pathologically confirmed GC (adenocarcinoma), and pathological wax blocks could be obtained; (II) patients with stage IV disease; (III) CPS PD-L1 negative; (IV) patients treated with a PD-(L)1 inhibitor combined with chemotherapy; (V) expected survival longer than 3 months.

In this study, the primary outcome was progression-free survival (PFS) and the exposure was the level of TILs. Observation was until death or end of follow-up (August 1, 2021), whichever came first. The follow-up data was obtained by e-mail, telephone or subsequent visit. This study has been approved by the Ethics Committee of Peking University Shenzhen Hospital (No. 2018022U). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Treatment

All patients received PD-(L)1 inhibitors plus chemotherapy, and the immunotherapy regimen was one of the following: pembrolizumab 200 mg, Q3W or sintilimab 200 mg, Q3W. As for chemotherapy, regimen was chosen from XELOX or SOX. The response was assessed by computed tomography (CT) every 2 cycles.

Principles for evaluating tumor response

To assess the response after treatment, complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were used according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (13). ORR means the proportion of patients who achieved a CR or PR in all patients, and similarly, DCR means the proportion of patients experienced CR, PR, or SD. PFS was considered as the duration from the beginning of treatment to the first occurrence of PD or death. Two inagedent radiologists or pathologists were assigned to assess the clinical characteristics.

Analysis of the TME

Multiplex immunofluorescence (mIF) was performed with Akoya OPAL Polaris 7-Color Automation IHC kit (Panovue, Inc., Beijing, China). Firstly, formalin-fixed paraffin-embedded (FFPE) tissue slides were deparaffinized in a BOND RX system (Leica Biosystems, Inc., Nussloch, Germany). Then, we incubated slides with primary antibodies targeting HLA-DR (Abcam, Massachusetts, US; ab215985, 1:500), CD68 (Abcam, ab213363, 1:1,000), PD-L1 (Cell Signaling Technology, Inc., Massachusetts, US; E1L3N, 13684S, 1:400), CD8 (Abcam, ab178089, 1:100), CD56 (Abcam, ab75813, 1:100), and pan-CK (Abcam, ab7753, 1:100). Then, the slides were incubated with secondary antibodies and bound with corresponding reactive opal fluorophores. Nucleic acids were stained with DAPI. Take one slide from each sample as negative control which were incubated with primary and secondary antibodies but not fluorophores and it was used to adjust autofluorescence. Multiple stained slides were scanned under Vectra Polaris Quantitative Pathology Imaging System (Akoya Biosciences, Ihc., Massachusetts, US) at 20 nm wavelength intervals from 440 to 780 nm with a fixed exposure time and an absolute magnification of ×200. Scans from the same slide were merged to get one single image. Quantitative analysis for multiple TILs was performed in inForm v.2.4.8 (Akoya Biosciences, Ihc., Massachusetts, US) with the imported images. Pan-CK was used to distinguish the border between tumor parenchyma and stroma. Stained immune cells per mm^2 and the ratio of stained cells to all nucleated cells were used to represented the level of $CD8^+$ T cells, M1 macrophages ($CD68^+$HLA-DR^+), M2 macrophages ($CD68^+$HLA-DR^-), $CD56^{bright}$ NK cells (bright, based on the relative expression of the surface marker CD56), and $CD56^{dim}$ NK cells (dim, based on the relative expression of the surface marker CD56).

Statistical analysis

SPSS 19.0 (SPSS1, Chicago, IL, USA) was used to perform the analysis of the data. Correct statistical methods were performed to address potential sources of bias. Normality test was performed on all variables, and all the normally distributed data were expressed as mean ± standard deviation. Two independent sample *t*-test was used to compare the differences between two groups of quantitative data. Test of the significance of difference for categorical data were performed by chi-square test or Fisher's exact test. Kaplan-Meier method was used for survival analysis, and the curves were compared by log-rank tests. The correlation between PFS and clinical characteristics was analyzed by Cox proportional hazards analysis. Confounding variables were identified preliminarily by univariate analysis and then the variables whose P value less than 0.1 in univariate analysis were examined by multivariate analysis. P value of less than 0.05 was considered to be statistically significant.

Table 1 Characteristics of the patients in the study

Characteristics	Overall (n=26)
Age	
≥60 years	14 (53.8%)
<60 years	12 (46.2%)
Sex	
Female	11 (42.3%)
Male	15 (57.7%)
Surgery	
Yes	4 (15.4%)
No	22 (84.6%)
Surgical margin status	
R0	2 (7.7%)
R1	2 (7.7%)
Primary tumor site	
Antrum	12 (46.2%)
Cardia	4 (15.4%)
Corpus gastricum	10 (38.5%)
Seroperitoneum	
Yes	3 (11.6%)
No	23 (88.4%)
Number of metastatic sites	
1	11 (42.3%)
>1	15 (57.7%)
Metastatic sites	
Celiac lymph nodes	5 (19.2%)
Left clavicle, celiac lymph nodes	4 (15.4%)
Liver, celiac lymph nodes	7 (27.0%)
Ovary, celiac lymph nodes	10 (38.4%)
Differentiation	
Moderate	5 (19.2%)
Poor	21 (80.8%)
Treatment line	
First line	23 (88.5%)
Second line	3 (11.5%)
Treatment regimen	
Sintilimab + chemo	16 (61.5%)
Other	10 (38.5%)
HER2	
Negative	20 (76.9%)
Positive	6 (23.1%)
MSI status	
MSI-H	1 (3.8%)
MSS	25 (96.2%)
Objective response	
CR	5 (19.2%)
PR	9 (34.6%)
SD	7 (26.9%)
PD	5 (19.2%)

Treatment regimens: sintilimab + chemo included sintilimab + SOX and sintilimab + XELOX. Others included pembrolizumab + trastuzumab + SOX, pembrolizumab + SOX, and pembrolizumab + irinotecan. MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable; CR, complete response; PR, partial response; SD, stable response; PD, progression disease.

Results

Patient characteristics

Twenty-six GC patients were enrolled ultimately (*Table 1*). They all received appropriate treatment in Peking University Shenzhen Hospital from July 2019 to January 2021 and resected tumors by biopsy were collected before recurrence treatment. The average follow-up time was 9.5 months. The longest follow-up time was 701 days, and the shortest was 44 days. A total of 53.8% of patients were above 60 years old and 57.7% of patients were male. Most of the patients' tumors were located in the antrum (46.2%). All of the patients were at the advanced stage. A total of 80.8% of patients had poor differentiation, and most of these patients were treated by sintilimab plus chemotherapy (61.5%), while others were treated by ICIs combined with chemotherapy in the first line (88.5%). A total of 23.1% of

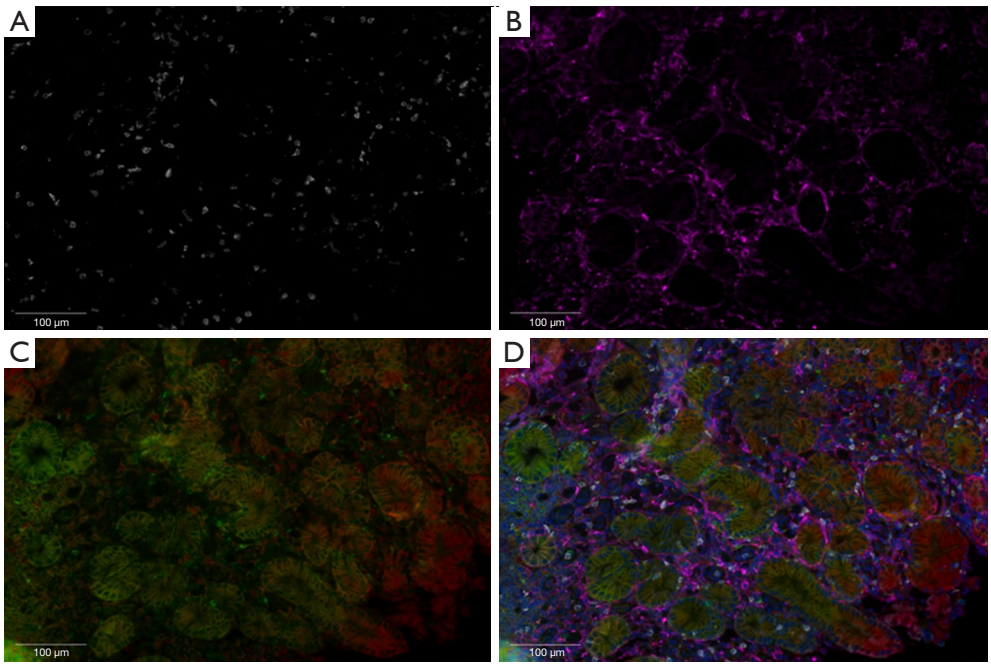

Figure 1 Representative images of mIF depicting cell infiltration in GC tissues. (A) Immunohistochemical staining of GC cells with CD8 antibody (white); (B) immunohistochemical staining of GC cells with CD56 antibody (purple); (C) immunohistochemical double-staining of CD68 (green) and HLA-DR (red) in GC tissues; (D) multiple stained slides were scanned by the Vectra Polaris Quantitative Pathology Imaging System. Representative merged images show the distribution of individual markers [CD8, CD56, CD68, HLA-DR, pan-CK (cyan), DAPI for nucleic acids (blue)], which allows the identification of different immune cell subtypes ($CD8^+$ T cells, M1/M2 macrophages, $CD56^{bright}$/$CD56^{dim}$ NK cells). Scale bar: 100 μm. GC, gastric cancer.

patients were positive for HER2.

Higher levels of intratumoral infiltrating $CD8^+$ T cells in CPS PD-L1-negative patients who responded to chemoimmunotherapy

As shown in *Table 1*, 5 (19.2%) patients were on CR, 9 (34.6%) patients were in PR, and 7 (26.9%) patients had SD based on central radiological assessment per RECIST version 1.1. In order to study the probable mechanisms of the better clinical outcome of responders (CR + PR + SD ≥6 months), peritumoral and intratumoral TILs were detected by mIF. Representative images of $CD8^+$ T cells, *M*1 macrophages, *M*2 macrophages, $CD56^{bright}$ NK cells, and $CD56^{dim}$ NK cells are shown in *Figure 1*. We quantified subtypes of TILs and compared the number and proportion of TILs between responders and non-responders. We found that the number and proportion of $CD8^+$ T cells were higher within tumors in responders (P=0.011), however, no statistical difference was observed (P=0.21) peritumorally between responders and non-responders (*Figure 2*). Furthermore, there were no significant differences for other types of TILs within tumors or peritumorally between responders and non-responders, probably because fewer subjects were enrolled. Our results suggested that patients with a better response to chemoimmunotherapy showed higher level of intratumoral $CD8^+$ T cells at baseline.

$CD8^+$ TILs are a predictive biomarker for PFS in CPS PD-L1-negative patients treated with chemoimmunotherapy

Because of the higher level of intratumoral $CD8^+$ T cells in responders, we examined whether $CD8^+$ TILs in CPS PD-L1-negative patients treated by chemoimmunotherapy could predict PFS. PFS was evaluated according to the number of intratumoral $CD8^+$ TILs. Firstly, receiver operating characteristic (ROC) curve analysis was performed to compare the discriminatory power for the patients' responses between different intratumoral infiltrating $CD8^+$ TIL percentages [area under the curve (AUC) =0.804,

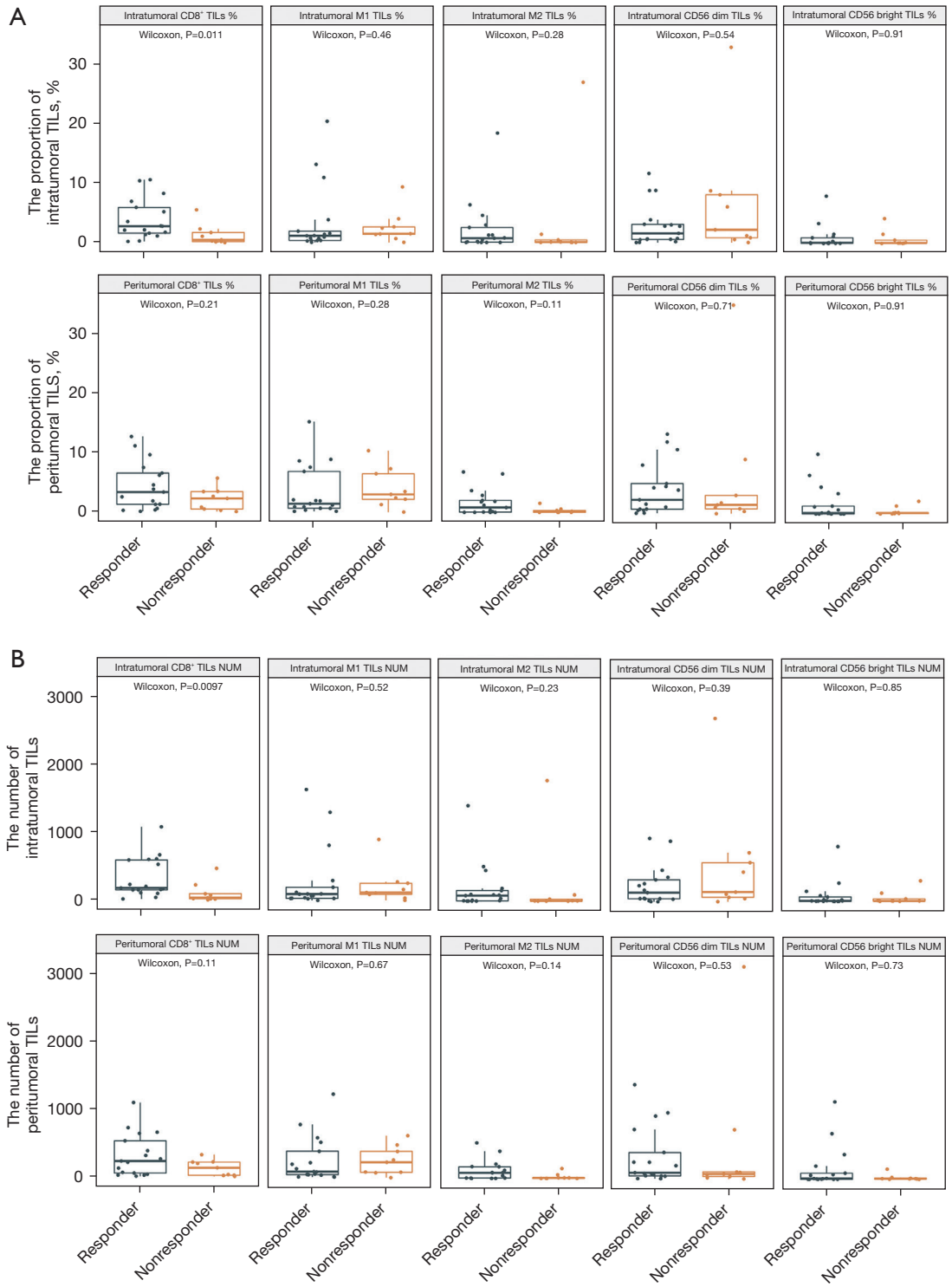

Figure 2 Intratumoral and peritumoral TILs in responders and non-responders. (A) The proportion (%) of intratumoral and peritumoral TILs between responders and non-responders was compared; (B) the number of TILs within the tumor or peritumorally between responders and non-responders was compared. Comparisons were performed with the Wilcoxon signed-rank test, and P value <0.05 was considered to be statistically significant. TILs, tumor-infiltrating lymphocytes.

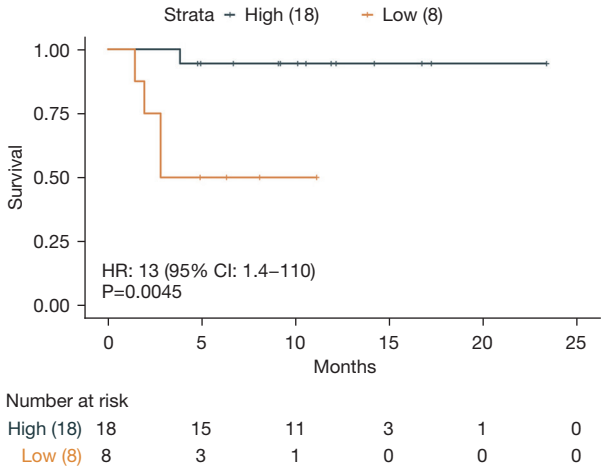

Figure 3 Kaplan-Meier analysis of PFS. Significantly prolonged PFS in patients in the HIGH subgroup compared to the LOW subgroup (P=0.0045). PFS, progression-free survival; High, high density of CD8+ TILs; Low, low density of CD8+ TILs; TILs, tumor-infiltrating lymphocytes.

95% CI: 0.621–0.987, P=0.012] and used to decide the cut-off value (Figure S1). Youden's index was used to separate high and low levels of intratumoral CD8+ TILs, and the cut-off point ends up being 1.085 (cut-off value 1.085%, sensitivity 88.2%, specificity 67%). According to the cut-off, intratumoral CD8+ TIL percentage was divided into categories designated as high (≥1.085%) and low (<1.085%). Tumors with low intratumoral CD8+ TIL percentage had worse PFS compared with tumors with high CD8+ TIL percentage, with a hazard ratio (HR) of 13 (95% CI: 1.4–110, P=0.0045; *Figure 3*).

The results of the univariate survival analysis for clinical characteristics including CD8+ TILs was shown in *Figure 4*. In the univariate analysis, clinical factors statistically associated with PFS were treatment regimen and CD8+ TILs. Treatment regimen and CD8+ TILs were significant predictors of PFS. Treatment with chemoimmunotherapy in the first line and high CD8+ TIL number were associated with better PFS, while age, gender, primary tumor site, and treatment were not associated with PFS. We adopted primary tumor site, treatment regimen, CD8+ TILs, and treatment as covariates for multivariate Cox proportional hazards analysis (*Figure 5*). CD8+ TILs were a significant predictor of PFS, while primary tumor site, treatment regimen, and treatment were not significant predictors of PFS. These results showed that CD8+ TILs are valuable biomarkers for CPS PD-L1-negative patients treated with chemoimmunotherapy, and CPS PD-L1-negative patients with high CD8+ TILs can benefit from chemoimmunotherapy.

Discussion

As expected, our study revealed the correlation between intratumoral infiltrating CD8+ T cells and treatment response in CPS PD-L1-negative patients. Patients with high CD8+ TILs instead of other cells in the TME experienced a higher ORR. According to the univariate or multivariate analysis, we derived intratumoral CD8+ TILs as a predictor of chemoimmunotherapy response in CPS PD-L1-negative patients.

PD-1/PD-L1 immune checkpoint has been demonstrated to be an essential regulator between tumor and T cells, and expression of PD-L1 in the surface of tumor cells may increase tumor immune escape (14). In recent years, PD-1/PD-L1 checkpoint blockade immunotherapy is becoming a new and powerful treatment. Based on their original mechanism, ICIs have become a significant therapeutic regimen for PD-L1-positive patients. The Keynote 059 trial demonstrated that pembrolizumab can be applied for third-line or subsequent therapy for PD-L1 positive GC patients (4). Meanwhile, a crucial clinical trial, CheckMate 649, demonstrated that chemoimmunotherapy showed better efficacy for PD-L1-positive patients in first-line treatment with ORR of 60% and median PFS of 7.7 months, comparing with chemotherapy (15). However, another phase 3 randomized clinical trial, Keynote 062, found that compared with chemotherapy, PD-L1-positive G/GEJ patients could not benefit from the regimen combined with pembrolizumab. Surprisingly, pembrolizumab showed excellent efficacy for MSI-H patients in Keynote 062 (16). This suggests that immunotherapy may have more mechanisms beyond PD-L1. Our retrospective study enrolled 26 patients with advanced G/GEJ cancer who were treated by chemoimmunotherapy as the first- or second-line treatment. They were all negative for PD-L1 (CPS <1) by IHC and almost all of them had microsatellite stable (MSS) resected tumor tissues. Importantly, most of these patients benefited from chemoimmunotherapy, with an ORR of 54.9%. In our cohort, the ORR with chemoimmunotherapy was almost the same as that in CPS PD-L1-positive patients (60% in CheckMate 649). This may have occurred due to fewer subject, however, we also explored the underlying mechanism of this result. We thus quantified the number and proportion of TILs between

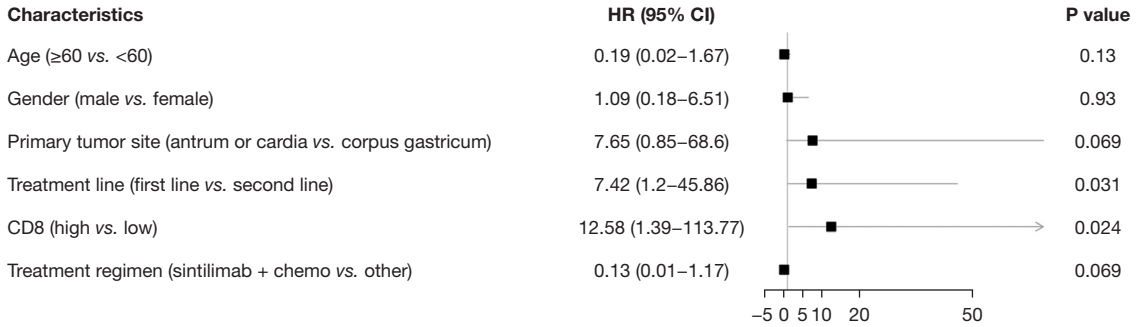

Figure 4 Univariate analysis of clinical characteristics as a predictor of time to progression shown as forest plots. CI, confidence interval; HR, hazard ratio.

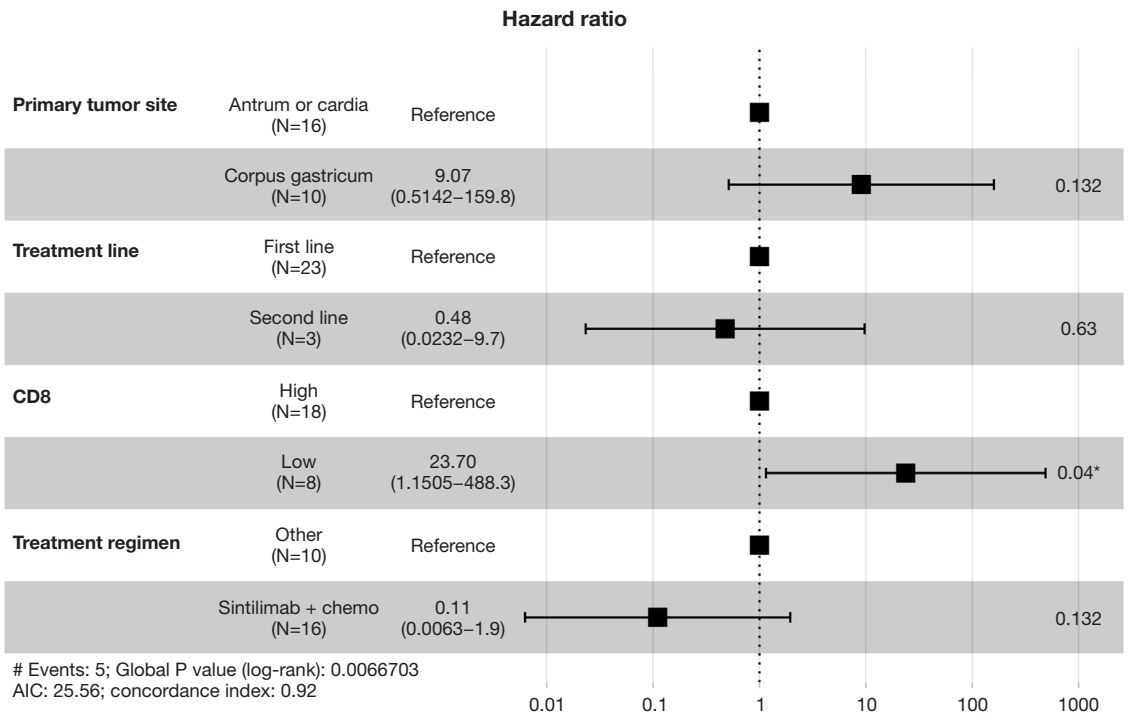

Figure 5 Multivariate analysis of clinical characteristics as a predictor of time to progression shown as forest plots. *, P value <0.05.

responders and non-responders and compared TILs by mIF. We found that higher intratumoral CD8$^+$ TILs were detected in responders. We enrolled 1 patient classified as MSI-H, which is considered as an important biomarker for immunotherapy based on Keynote 158 (17). We believe that this patient had little impact on the outcome, as MSI status was a biomarker used in the second-line therapeutic regimen and Keynote 158 only included 24 patients (10.3%) with advanced G/GEJ of the 233 enrolled pan-cancer patients, requiring further clinical validation of MSI (17). We raised the possibility that patients negative for PD-L1 do not have to undergo MSI, TMB, or other tests except for CD8$^+$ T cell infiltration, which will be of great convenience for clinical practice to determine whether to perform immunotherapy or not.

TME is related to cancer development, progression, and cancer-related immune reactions, and has thus emerged as focus of attention in cancer research (18,19). It was reported that some TILs are associated with the prognosis of GC patients (20,21). Thompson *et al.* reported that high CD8$^+$

T cells in the tumor showed better prognosis in G/GEJ patients with significantly prolonged PFS or OS (22). Kawazoe *et al.* conducted a retrospective analysis of a cohort of 487 GC patients and found that patients with high $CD8^+$ T cells in tumors had increased OS (23). This may be because $CD8^+$ T cells are considered to be dominating anti-tumor force in immune system. After binding to tumor cells, $CD8^+$ T cells produce perforin and other cytotoxins that kill cancer cells but leave normal cells alive. The tumor-driven microenvironments offer the indispensable conditions for tumor survival, which can decrease or weaken $CD8^+$ T cells (24). Thus, tumors with low $CD8^+$ T cells may be more malignant and patients may experience poor prognosis. As for the function of intratumoral $CD8^+$ TILs during immunotherapy, Wang *et al.* reported that $CD8^+$ T cells in the tumor can regulate tumor ferroptosis via Fas-Fas ligand pathways (25). It was also reported that 2 subsets of cells were present in $CD8^+$ T cells, and they can mediate the response of adoptive cell immunotherapy against human cancer (26). In the current study, we demonstrated that $CD8^+$ TILs are predictive biomarkers for response to chemoimmunotherapy in CPS PD-L1-negative patients through univariate or multivariate analysis, which may be based on the mechanisms mentioned above. Although different therapeutic regimens showed stratification in the univariate analysis with $P \leq 0.05$, multivariate Cox proportional hazards analysis demonstrated that $CD8^+$ TILs were the unique significant predictor of PFS for CPS PD-L1-negative patients treated by chemoimmunotherapy. Because the location of the tumor and the treatment regimen may have an impact on the outcome, multivariate analysis took these factors into account. Furthermore, no matter what the immunotherapeutic or chemotherapeutic agent the patients take, $CD8^+$ TILs are still a powerful predictor.

Other subtypes of TILs are also important components of the immune microenvironment. For example, NK cells can recruit conventional type 1 dendritic cells into the TME to promote cancer immune control (27). Further research showed that they can be subdivided into 2 major subtypes, namely $CD56^{dim}$ cytotoxic and $CD56^{bright}$ immunoregulatory NK cells, which may produce different effects in the TME (28). M1 macrophages can kill tumor cells and inhibit tumor cell growth by phagocytosis and by mediating the Th1 response. M2 macrophages promote tissue repair, angiogenesis, and immunosuppression by producing cytokines and mediating the Th2 response, further contributing to tumor progression (29). However, we found no difference in these subsets between responders and non-responders within the tumor or peritumorally. This is probably because their role in the TME of gastric cancer may be less pronounced or the number of subjects enrolled resulted in a non-significant trend.

As mentioned above, this retrospective study included a small number of patients, and the results need to be further confirmed in a cohort with more subjects. The TME contains more than T cells, B cells, macrophages, or NK cells. The role of other immune cells in the effect of chemoimmunotherapy remains to be determined. However, this study provides a good foundation for expanding the number of subjects in our next research.

In conclusion, we found increased intratumoral $CD8^+$ T cells in CPS PD-L1-negative patients who responded to chemoimmunotherapy. Intratumoral $CD8^+$ T cells could be served as a potential predictive biomarker for CPS PD-L1-negative patients in the treatment of chemoimmunotherapy.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-644/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-644/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-644/coif). All authors report that this work was supported by the Shenzhen Science and Technology Innovation Commission Project (JCYJ20190809100005672, ZDSYS20190902092855097, KCXFZ20200201101050887), General Program (JCYJ20210324105609024) and Shenzhen Sanming Project (SZSM201612041). YC and SW were employed by 3D Medicines Inc. The authors have no other conflicts of

interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study has been approved by the Ethics Committee of Peking University Shenzhen Hospital (No. 2018022U). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Signed written informed consent forms were obtained from patients prior to first dose of therapeutic regimen.

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Supplementary

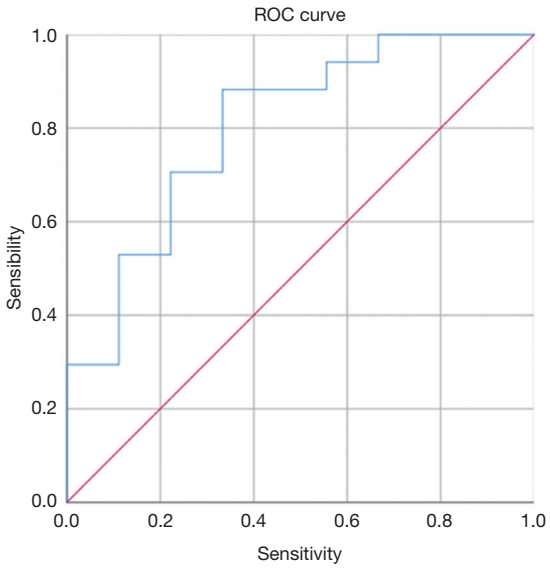

Figure S1 ROC curve analysis was conducted to obtain the cut-off value in terms of the maximum of Youden's index. ROC, receiver operating characteristic.

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