Clinical significance of peripheral TCR repertoire profiling and individualized nomograms in patients with gastrointestinal cancer treated with anti-PD-1 antibody

Jing Wu
Fudan University

Yiyi Yu
Fudan University

Shilong Zhang
Fudan University

Pengfei Zhang
Fudan University

Shan Yu
Fudan University

Wei Li
Fudan University

Yan Wang
Fudan University

Qian Li
Fudan University

Binbin Lu
Shanghai Dunwill Medical Technology Co., Ltd

Limeng Chen
Shanghai Dunwill Medical Technology Co., Ltd

Chonglin Luo
Shanghai Dunwill Medical Technology Co., Ltd

Haixiang Peng
Shanghai Dunwill Medical Technology Co., Ltd

Tianshu Liu
Fudan University

Yuehong Cui (✉ cui.yuehong@zs-hospital.sh.cn)
Fudan University

Research Article

Keywords: T cell receptor repertoire, immune-related adverse events, Anti-PD-1 treatment, gastrointestinal cancers, DE50
Abstract

Background

Immune checkpoint inhibitors (ICIs) have significant clinical benefit for a subset of patients with gastrointestinal cancers including esophageal cancer, gastric cancer and colorectal cancer. However, it is difficult to predict which patients will respond to immune therapy or induce immune-related adverse events (irAEs). This study was initiated to determine if peripheral T-cell receptor (TCR) repertoire profiling could predict the clinical efficacy of anti-PD-1 treatment, while also predict adverse events.

Methods

Blood samples from 31 patients with GICs were collected before anti-PD-1 antibody treatment initiation. The clinical significance of TCR repertoire profiling from PBMCs was evaluated in all the enrolled patients. A highly predictive nomogram was set up based on peripheral TCR repertoire profiling. The performance of the nomogram was assessed by receiver operating characteristic (ROC) curve, concordance index (C-index), and calibration curves, and decision curve analysis (DCA) was used to assess its clinical applicability.

Results

Compared to non-responders (PD), the DE50 scores were significantly higher in responders (SD and PR) (P = 0.018). There was a trend that higher DE50 at baseline was associated with the occurrence of adverse events, but it did not reach statistical significance (P = 0.1779). Patients with a high DE50 score showed better progression-free survival (PFS) than those with a low DE50 score (P = 0.0022). The multivariable Cox regression demonstrated that high DE50 and low PLR were significant independent predictors for better PFS when treated with anti-PD-1 antibody. Furthermore, a highly predictive nomogram was set up based on peripheral TCR repertoire profiling. The AUCs of this system at 3-, 6- and 12-month PFS reached 0.825, 0.802, and 0.954, respectively. The nomogram had a C-index of 0.768 (95% CI: 0.879 – 0.658). Meanwhile, the calibration curves also demonstrated the reliability and stability of the model.

Conclusions

High DE50 scores were predictive of a favorable response and longer PFS to anti-PD-1 treatment in GIC patients. The nomogram based on TCR repertoire profiling was a reliable and practical tool, which could provide risk assessment and clinical decision-making for individualized treatment of patients.

Introduction

Gastrointestinal cancers (GICs) including esophageal, gastric, and colorectal cancers are common malignancies which are often diagnosed at a late stage [1]. The lack of effective early diagnosis and treatment strategies of gastrointestinal cancer result in a poor prognosis. Currently, immunotherapy such as anti-programmed death 1 (PD-1) antibody has gained a significant value in the treatment of some gastrointestinal cancers and has been incorporated into treatment regimens [2]. However, a large number of patients do not respond to these treatment
approaches, and toxicity may be substantial. Thus, it is of great significance to identify a biomarker to predict patients who will respond to anti PD-1 therapy and then help optimizing patients' stratification for the best possible therapeutic outcome.

T cell receptor (TCR) is a surface molecule of T cells that could specifically recognize antigens presented by the major histocompatibility complex (MHC) and mediate immune response [3]. It is a heterodimer, the majority (~95%) of which consists of an α-chain and a β-chain in human [4]. The complimentary determining region 3 (CDR3) of TCRβ chain is the highly variable component of TCRs and is critical for the specificity of each T cell clone for antigen recognition [5]. CDR3 polymorphisms generated by random rearrangements of the variable (V), diversity (D), and joining (J) gene segments, determines TCR diversity. Random insertion or deletion of non-template nucleotides in V-D and D-J junction regions during rearrangement increase TCR diversity. Theoretically, the ability of the immune responses to a variety of different antigens depends on a large repertoire of unique TCR.

Several studies have shown that TCR repertoire diversity plays an important role in tumor immunity [6–9]. Analysis of TCR repertoire profiling can be used to reflect the immune responses for some patients and may help predict the clinical outcomes of anti-PD-1 therapy. In this study, we used the Oncomine TCR Beta-LR Assay to detect the possible V-J rearrangement in the CDR3 region of the TCRβ chain in peripheral blood samples from GIC patients. The aim of this study was to assess whether the TCR repertoire diversity evenness (DE50), before treatment initiation, could predict which subpopulation of patients was more likely to respond to anti-PD1 therapy. We showed that patients with a high DE50 score could benefit from anti-PD-1 treatment, but may be prone to develop immune-related adverse events (irAEs). These findings would help to characterize the TCR repertoire profiling in the tumor microenvironment in GICs. Furthermore, we suggested that the quantification of TCR repertoire diversity in the peripheral blood, prior to treatment initiation, could represent a predictive biomarker to guide the use of immunotherapies to the most appropriate GIC patients.

Methods

Patients

Peripheral blood samples were obtained from a total of 31 GICs patients between April 2019 and January 2021, including esophageal cancer patients (n = 7), gastric cancer patients (n = 20) and colorectal cancer patients (n = 4). The study enrolled GIC patients with histologically confirmed unresectable disease or metastatic disease. Patients included in the study ranged from 19 to 80 years of age with Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. Patients who had previously received immunotherapy were excluded. All patients were followed up and the median duration of patient follow-up was 11.9 months. Peripheral blood was collected and laboratory indices, including CEA levels, neutrophil, platelet, lymphocyte and leukocyte counts were recorded within 7 days prior to the beginning of the first cycle of anti-PD-1 treatment. All subjects were recruited from the Fudan University Shanghai Cancer Center and proved by pathologic examination. Cross-sectional imaging of the chest, abdomen, and pelvis was obtained every three cycles. The objective response rate (ORR) and PFS were evaluated independently by physicians and two experienced radiologists according to immune-related response criteria (irRECIST) for each patient. Clinical severity of irAEs was graded according to Management of immunotherapy-Related Toxicities, Version 1.2021.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll-PaqueTM PLUS (GE healthcare, Cat. No. 17-1440-03). The fresh PBMCs were lysed with Lysis buffer using the
MagMAX™ mirVana™ Total RNA Isolation Kit (Thermo Fisher Scientific Cat. No. A27828) and stored at -80°C until further use. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Fudan University Shanghai Cancer Center. All patients signed the written informed consents before participation.

**TCRβ sequencing**

For the Ion Torrent-based approach, RNA was extracted from PBMC using the MagMAX™ mirVana™ Total RNA Isolation Kit (Thermo Fisher Scientific Cat. No. A27828). Purified RNA samples were quantified using Qubit RNA HS Assay Kit (Thermo Fisher Scientific Cat. No. Q32852). The Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit were used to quantify and evaluate RNA integrity. 50 ng of total RNA was reverse transcribed using SuperScript IV VILO Master Mix (Thermo Fisher Scientific Cat. No. 11756050). For each sample, 25 ng cDNA was amplified using the Oncomine TCR Beta-LR Assay (Thermo Fisher Scientific Cat. No. A35386), and protocol as described in Oncomine™ Human Immune Repertoire User Guide MAN0017438 Revision C.0. Libraries were purified with Agencourt AMPure XP beads (Beckman Coulter Cat. No. A63880), washed with 70% ethanol, and eluted in 50 µL Low TE buffer. Resulting library samples were diluted and quantified using the Ion Library Quantitation Kit (Thermo Fisher Scientific Cat. No. 4468802), then diluted to 25 pM with Low TE buffer. Equal volumes from 6–7 samples at a time were pooled together for sequencing on one Ion 530 chip, followed by analysis via Ion Reporter version 5.14.

**Sequencing Data Analysis**

The analysis process was shown in Fig. S1. The raw sequencing data (.bcl) generated from the Ion Torrent S5 XL sequencer were demultiplexed and preprocessed using BaseCaller plugin (5.10) to produce sample-specific UBAM files. Briefly, the sequencing adaptors and target-specific primers were trimmed, before further trimming of the reads to retain only high-quality data (Q20 or higher). The run result files were then transferred to the Ion Reporter™ Software 5.14 using Ion Reporter Uploader plugin, followed by post-processing to filter low quality and off-target reads, remove or correct error containing reads, generate report for VDJ rearrangements and perform secondary analysis of repertoire features, including evenness and Shannon diversity index. Shannon diversity index was calculated as below:

$$\text{Shannondiversityindex} = -\sum_{i=1}^{n} p_i \log_2 (p_i)$$

where \(p_i\) was the frequency of clonotype \(i\) for the sample with \(n\) unique clonotypes. Evenness was a normalized Shannon diversity index as indicated below [10, 11]:

$$\text{evenness} = \frac{\text{Shannon diversity}}{\log_2 (n)}.$$

All the analyses mentioned above (including Shannon diversity index and evenness) used the default parameters of Ion Torrent S5 XL Server or Ion Reporter™ Software 5.14, two well-established commercial software. As for DE50 calculation, we used our in-house workflow adapted from a previous report [12]:

$$\text{DE50} = \frac{\text{no. of the most frequent clonotypes accounting for 50}\% \text{ of the sum of each clonotype's frequency}}{\text{total no. of clonotypes}}$$
A schematic graph is provided to help understand the meaning of DE50 in Fig.S2.

**Statistical Analysis**

SPSS 26.0 software and GraphPad Prism 8.0 were used to perform the analysis. Statistical significance was calculated by two-sided t-test (95% confidence interval). The primary endpoints included ORR and PFS, and the secondary end-point was safety. The Kaplan-Meier was used to estimate progression-free survival (PFS). Correlations between variables, such as TCR repertoire diversity, curative effect or adverse events, were analyzed using Mann-Whitney U test. Independent predictive factors for clinical efficacy were investigated by univariable and multivariable logistic regression models. Univariable logistic regression models with the objective response (OR) status as the binary outcome and Sex, Age, Evenness, Shannon diversity, DE50, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), or CEA as the predictor were built. According to the P-value of the outputs from the univariable logistic regression models, a multivariable logistic regression model was built with the OR status as the binary outcome and Shannon diversity, DE50, and CEA as the predictors. Cox proportional-hazards regression models were used to evaluate the relationship between PFS and each of the same variables as used in the univariable logistic regression models. A multivariable Cox regression analysis was then performed with selected variables DE50, PLR, and CEA according to the P-value of the outputs from the univariable analyses. Both the univariable and the multivariable logistic regression were run using R with method= "glm", family= "binomial" and link="logit". The Cox Proportional-Hazards Regression was fit in R with the survival package and its coxph function. All the forest plots were drawn in R with package "forestplot". Variables with p < 0.2 in univariable logistic regression models were included in a multivariable logistic regression model. ***p-value < 0.001, ** p-value < 0.01, * p-value < 0.05.

**Results**

**Characteristics of the patients**

A total of 31 patients with GICs treated with PD-1 inhibitors were enrolled in this study. The demographic and baseline characteristics were depicted in Table 1. The majority of the patients were male (74.2%) with a median age of 64 years (range 39–79 years) and an ECOG PS of 0–2 at baseline. Of the local GIC therapies, 36% of patients received anti-PD-1 antibody combined with anti-angiogenics, 19% received anti-PD-1 antibody combined with chemotherapy and 45% received anti-PD-1 monotherapy. For data analysis, partial response (PR, n = 5) and stable disease (SD, n = 14) were considered as treatment responders whereas progression disease (PD, n = 12) groups are defined as non-responders. Survival plot for PFS was shown in Fig.S3.
Table 1
The demographic and clinical characteristics of 31 patients with GICs

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (74.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>Age, median [range]</td>
<td>64 (39–79)</td>
</tr>
<tr>
<td>Tumor types</td>
<td></td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>7 (22.6%)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>20 (64.5%)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>ECOG score</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>23 (74.2%)</td>
</tr>
<tr>
<td>2</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>Treatment line</td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>19 (61.3%)</td>
</tr>
<tr>
<td>≥ 3</td>
<td>12 (38.7%)</td>
</tr>
<tr>
<td>PD-L1 agents</td>
<td></td>
</tr>
<tr>
<td>22C3</td>
<td>8 (62%)</td>
</tr>
<tr>
<td>E1L3N</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>PD-L1 expression</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6 (19.4%)</td>
</tr>
<tr>
<td>Positive*</td>
<td>7 (22.6%)</td>
</tr>
<tr>
<td>Missing</td>
<td>18 (58.0%)</td>
</tr>
<tr>
<td>CEA</td>
<td></td>
</tr>
<tr>
<td>&lt;5ug/L</td>
<td>18 (58.1%)</td>
</tr>
<tr>
<td>≥ 5ug/L</td>
<td>13 (41.9%)</td>
</tr>
</tbody>
</table>

*PD-L1 positivity was defined as CPS ≥ 1

Profiling Of Tcrβ Cdr3 In All Patients

A total of 31,531,729 TCRβ CDR3 aa sequence reads were produced from PBMCs of the 31 patients, with an average of 1,017,153 TCRβ CDR3 aa sequence reads per sample. Fifty-three distinct Vβ gene segments, 13 various
Jβ gene segments, and 664 unique V-J pairs were identified in all of the samples. First, we examined the effects of pre-existing TCR repertoire characteristics from baseline PBMC on patient response to anti-PD-1 therapy. For the patient response outcomes, we classified patients into three groups based on the best response, that is, CR/PR, SD, and PD. As shown in Figure S4, we found that the number of distinct Vβ gene segments per sample in SD + PR group was slightly higher than that in PD group (Mann-Whitney Test, P = 0.0236). The most frequent Vβ gene segments in all of the samples were TRBV20.1 (8.17% ± 0.38%), TRBV28 (6.87% ± 0.50%), TRBV7.2 (6.24% ± 0.45%), TRBV5.1 (5.54% ± 0.33%) and TRBV29.1 (5.32% ± 0.32%) (Fig.S4A). Thirteen various Jβ gene segments were detected in every single sample, with the most frequent Jβ gene segments being TRBJ2.1 (18.17% ± 0.49%), TRBJ2.7 (16.78% ± 0.65%), TRBJ2.3 (12.11% ± 0.60%), TRBJ1.1 (11.29% ± 0.45%), TRBJ2.5 (9.33% ± 0.41%) (Fig.S4B).

Tcr Diversity Is Predictive For Icis Benefit As Well As Adverse Events

In order to identify the potential predictors of clinical outcomes of anti-PD-1 therapy, we also analyzed the correlation between diversity indices (Evenness, Shannon_diversity and DE50) and response to treatment using Chi-square test (Fig. 1). Evenness value in responders (PR + SD) was higher than that in non-responders (0.8875 vs. 0.8349, P = 0.0392) (Fig. 1A). However, no significant difference in Shannon_diversity was observed between the two groups (12.56 vs. 11.72, P = 0.1905) (Fig. 1B). DE50 (diversity evenness score) was used to comprehensively evaluate TCR repertoire diversity. Higher DE50 score corresponds to less clonality and higher TCR. We further investigated the DE50 scores, and observed a higher DE50 score in responders compared to non-responders (0.09334 vs. 0.04254, P = 0.018) (Fig. 1C).

To further confirm the relationship between baseline clinical parameters (including TCR diversity) and anti-PD-1 therapy response, univariable and multivariable logistic regression analyses were performed using median as the cut-off of the evenness (5.2) and Shannon diversity (3.62), respectively. The univariable model identified baseline DE50 (OR = 0.15, 95% CI = 0.03–0.78, P = 0.024) as the only variable significantly associated with response to therapy (Fig. 2A). in the multivariable logistic regression analyses where variables with P values < 0.2 were included, patient with higher DE50 levels tended to enjoy better response to anti-PD-1 therapy, although difference did not reach a significant level (OR = 0.21, 95% CI = 0.03–1.32, P = 0.096) (Fig. 2B).

Since the occurrence of irAEs might be correlated with better clinical outcome, we wondered whether gastrointestinal cancer patients with irAEs have a lower risk for progression when treated with anti-PD-1 therapy. Therefore, we subsequently divided the patients into irAE group (excluding ≥ grade 2 ICI-related myocarditis and pneumonia; n = 11) and non-irAE group (n = 16). The results indicated that patients who developed irAEs had a better PFS than those who do not have irAEs (HR = 0.3985, p = 0.0272) (Fig. 3A). There was no significant difference between irAE group and non-irAE group in the baseline TCR diversity such as Evenness (p = 0.4809) (Fig. 3B), Shannon_diversity (p = 0.5765) (Fig. 3C) and DE50 (p = 0.1779) (Fig. 3D). However, we observed a trend toward increased DE50 scores in irAE group (Fig. 3D). These results suggested that the DE50 scores might contribute to slightly higher risk of irAEs and improvement in PFS with anti-PD-L1 therapy.

De50 Was An Independently Prognostic Factor In Gastrointestinal Cancer Treated With Anti-pd-1 Antibody
To further explore the relationship between the baseline TCR diversity and PFS, Kaplan-Meier survival analyses were performed. In the included cohort, all the patients were divided into high- and low-TCR diversity groups using median as the cut-off the evenness (0.89) and Shannon diversity (12.24), respectively. As a result, the Kaplan-Meier analysis showed that the patients with either high-evenness diversity or Shannon diversity failed to enjoy a favorable PFS ($p = 0.77$ and $p = 0.53$, respectively) (Fig. 4A-B). However, patients with higher baseline DE50 had significantly longer PFS than patients with low baseline DE50 ($p = 0.022$) (Fig. 4C). Thus, in order to assess the independent predictive value of DE50, univariable and multivariable Cox regression analyses were performed. In the Cox proportional hazards regression analysis, we discovered that baseline DE50 ($HR = 0.24$, 95% CI = 0.09–0.64, $P = 0.005$) and PLR ($HR = 2.46$, 95% CI = 1.04–5.79, $P = 0.040$) as the only two variables significantly associated with PFS (Fig. 5A). After adjustment for age, gender, tumor site and histological type, to our surprise, DE50 ($HR = 0.22$, 95% CI = 0.08–0.64, $P = 0.006$) and PLR ($HR = 2.73$, 95% CI = 1.15–6.45, $P = 0.022$) were found to be independent factors associated with PFS (Fig. 5B). Furthermore, DE50 (C-index = 0.670), and PLR (C-index = 0.628) had higher discrimination ability in the prediction of PFS than the remaining factors, including CEA with a C-index of 0.577.

**Baseline DE50 levels, combined with PLR and CEA, was able to better predict PFS for patients with gastrointestinal cancer treated with anti-PD-1 antibody**

The above results indicated that the level of DE50 and PLR were independent prognostic factors for improvement of PFS in patients with gastrointestinal cancer treated with anti-PD-1 antibody. Since serum CEA is recommended as a tumor marker in GIC for tumor detecting and monitoring response to therapy. A nomogram for prediction of peritoneal metastasis probabilities, which included DE50 levels, PLR and CEA were constructed (Fig. 6). ROC curve was used to analyze the power of nomogram to predict PFS among gastrointestinal cancer patients treated with anti-PD-1 antibody. According to the ROC analysis, the AUCs of this system at 3-, 6- and 12-month PFS reached 0.825, 0.802, and 0.954, respectively (Fig. 7A). The nomogram had a C-index of 0.768(95% CI: 0.879 – 0.658). Meanwhile, the calibration curves demonstrated considerable agreement between the nomogram predicted and actual survival at 3-, 6- and 12-month PFS, respectively (Fig. 7B). These validations suggesting that this model can accurately predict the possibility of PFS among gastrointestinal cancer patients treated with anti-PD-1 antibody. In addition to address the accuracy, DCA was introduced to evaluate the clinical utility of this nomogram. Figure 7C showed that the established nomogram had favorable clinical applicability in predicting PFS.

Furthermore, to establish a risk stratification system based on our nomogram, we calculated the total score for each patient in the training cohort, and then stratified the patients according to the median of total scores into three subgroups: low-risk group and high-risk group. Kaplan-Meier analysis indicated that the survival time of patients in the high-risk group was much shorter than that of the low-risk group (Fig. 8A). Furthermore, the risk score reached an AUC value of 0.825, 0.802, and 0.954 for 3-, 6- and 12-month PFS, respectively (Fig. 8B).

**Discussion**

Recently, anti-PD-1 antibodies have been approved for the treatment of gastrointestinal cancer, exhibiting encouraging outcomes [2, 13–14]. However, patients' responses are often unpredictable. Few reliable prognostic and predictive markers are available that identify patients who might benefit from PD-1 inhibitors [13]. Programmed death-ligand 1 (PD-L1) expression has been considered as selection criteria of cancer patients for immune checkpoint inhibitors [15]. However, it is still an imperfect biomarker because some patients without PD-L1 expression also show a clinical response when treated with anti-PD-1 inhibitors [16, 17]. In addition, the IHC
scoring criteria of PD-L1 were not uniform across different antibody manufacturers. Tumor mutation burden (TMB) seems to be a valuable predictor of response to immunotherapy for gastrointestinal cancer [18–21]. However, there's still no consensus on the definition of TMB-high and TMB-low. Large-scale and phase III clinical studies would be needed to identify the cut-off values of TMB for various cancer types. Microsatellite instability high (MSI-H) or deficiency of mis-match repair (dMMR) status was found to be a valuable predictive biomarker for response to immunotherapy [13, 17, 22–24]. However, given the relatively low prevalence of MSI-H, MSI-H cannot satisfy the need for the majority of gastrointestinal cancer. For example, the incidence of MSI-H in small intestinal carcinoma was 4.6%, in colorectal cancer was 4.5% and in gastric cancer was 3.4%. The other challenge for all those biomarkers were the acquisition of adequate tumor sample for detection. Thus, the discovery of novel biomarkers that would predict the efficacy of PD-1 inhibitors is crucial.

T-cell-mediated cellular immunity plays an important role in antitumor responses to immunotherapy [25]. The TCR is an essential membrane protein that can recognize specific antigens on tumor and participate in the activation of T-cells. Monitoring TCR repertoire diversity may be helpful in assessing the immune therapy efficacy and prognosis. The antigen-specificity of tumor-reactive T-cells in peripheral blood and tumor tissue seem to be very much alike [26, 27]. Liquid biopsy is non-invasive and has become an alternative choice in clinical practice. Recent advances in next-generation sequencing (NGS) technology provide a detailed and complete description of multiple TCR clones in order to determine the diversity of TCR in the repertoire. Here, our study sought to use multiplex PCR amplification and deep sequencing of CDR3 region to assess TCR repertoire profiling in the peripheral blood.

Previous studies have demonstrated that pre-treatment TCR repertoire diversity might be associated with clinical outcome of immune therapy [27–33]. However, the results of these studies are sometimes not consistent or even contradictory. These results highlight the need for more detailed studies regarding some particular tumor type. To our knowledge, little is known about clinical significance of peripheral TCR repertoire profiling in patients with gastrointestinal cancer treated with anti-PD-1 antibody. In the present study, we demonstrated that higher DE50 scores (combinatorial diversity evenness of the TCR repertoire) were associated with better prognosis and increased response to anti-PD-1 inhibitors in gastrointestinal cancer. Importantly, this association was independent of other clinical factors such as age, gender, CEA. A possible explanation for these results is that higher TCR diversity may provide greater opportunities for our adaptive immune system to recognize antigens, increasing the possibility of more tumor-specific T cells to be activated to eliminate tumor cells [29, 31, 34]. Conversely, low TCR diversity may be associated with the impaired immune status that contribute to poor immunotherapy response [29, 31, 35].

Recently, irAEs have attracted increasing attention. It is reported that clinical efficacy of immunotherapy was associated with the occurrence of irAEs in various cancers [36, 37]. Our studies demonstrated that gastrointestinal cancer patients with high DE50 score showed favorable outcome than those with low DE50 score. Higher DE50 at baseline was associated with the occurrence of adverse events after anti-PD-1 treatment. However, it did not reach statistical significance probably due to the small sample size.

In order to obtain better predictive power, a combined model based on peripheral DE50 score and PLR is developed in this study at the pre-treatment time point. PLR is a routinely systemic inflammatory marker and may be a significant factor for predicting survival and response to therapies in cancer [38–40]. Thrombocytosis and the release of platelet-derived chemokines in the tumor microenvironment may promote tumor progression [41]. In contrast, lymphocytes are associated with immune surveillance and prevent development of malignancy [42]. Therefore, thrombocytosis and lymphocytopenia have been suggested as negative prognostic markers in various
on the basis, we speculated that the biomarker combining PLR and DE50, may better reflect the information of inflammatory/immune system status and predict the prognosis of GICs patients. The result suggested that such combinations of two different biomarkers could help to more accurately identify patients who may benefit from anti-PD-1 treatment strategies in gastrointestinal cancer. Further prospective research is needed to validate and refine the model.

Conclusions

TCR repertoire profiling could serve as a useful indicator for predicting treatment response to anti-PD-1 immunotherapy and prognosis in gastrointestinal cancer. In addition, DE50 was an independent predictive factor for PFS according to multivariable Cox regression analysis. Notably, these findings could be utilized to direct future immunotherapy if validated in further prospective studies. Still, there are some limitations in this study. First, this is a small study with a limited number of patients. Second, the study only focused on the β chain of the TCR, which can not entirely represent the characteristics of the whole TCR repertoire. The third limitation in this study was a lack of dynamic changes of TCR repertoire profiles during tumor treatment and evolution as well as a lack of longer follow-up. Due to the short duration of follow-up of the patients, analysis of OS could not be performed. Further study with a larger cohort and longer follow-up period would be required to validate these findings thus provide significant insight in the immunotherapy of gastrointestinal cancer.

Abbreviations

ICIs: immune checkpoint inhibitors; irAEs: immune-related adverse events; TCR: T-cell receptor; PD-1: programmed death 1; ROC: receiver operating characteristic; C-index: concordance index; DCA: calibration curves, and decision curve analysis; PFS: progression-free survival; GICs: gastrointestinal cancers; MHC: major histocompatibility complex; CDR3: complimentary determining region 3; ECOG: Eastern Cooperative Oncology Group; ORR: objective response rate; irRECIST: immune-related response criteria; PBMCs: peripheral blood mononuclear cells; NLR: neutrophil-lymphocyte ratio; PLR: platelet-lymphocyte ratio; PR: partial response; SD: stable disease; PD: progression disease; PD-L1: programmed death-ligand 1; TMB: tumor mutation burden; MSI-H: microsatellite instability high; dMMR: deficiency of mis-match repair; NGS: next-generation sequencing.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhongshan Hospital of Fudan University. Written informed consent was obtained from individual or guardian participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.
Competing interests

The authors declare no conflicts of interest.

Funding

This research was supported by The Science and Technology Commission of Shanghai Municipality, 19ZR1409500, and Study on the prevention and control of major chronic non-infectious diseases, National Key Research and Development Plan of China, 2017YFC1308900.

Data Availability Statement

The reviewers and editors can access sequencing data from the following site: https://dataview.ncbi.nlm.nih.gov/object/PRJNA752054?reviewer=oi9oq1p92j2u3gqspv6jv1nhrc

Author contributions

Each author participated sufficiently in the work to take responsibility for appropriate portions of the content. JW wrote the first draft of manuscript. JW, YY, TL, YC, CL and HP conceived and designed the experiments. SZ, PZ, SY, WL and BL performed the experiments. SZ, YW, QL and CL analyzed the data. All authors read and approved the final manuscript.

Acknowledgements

We gratefully acknowledge the support from Medical Oncology Department of Zhongshan Hospital. We also give our sincere thanks to the patients who participated in this study.

References


Figures
Figure 1

Baseline TCR diversity in responders and non-responders to anti-PD-1 treatment. (A) Evenness value; (B) Shannon diversity; (C) DE50 value.
Figure 2

Forest plots of univariable (A) and multivariable (B) logistic regression analyses.
Figure 3

The PFS and baseline TCR diversity between irAE and non-irAE groups. (A) FS probability; (B) Evenness value; (C) Shannon diversity; (D) DE50 value.
Figure 4

Kaplan-Meier curves of PFS for gastrointestinal cancer patients based on TCR diversity. (A) Evenness value; (B) Shannon diversity value; (C) DE50 value.
Figure 5

Forest plots of univariable (A) and multivariable (B) Cox regression analyses.
Figure 6

Nomogram for predicting PFS at 3-, 6-, and 12-month of gastrointestinal cancer patients.
Figure 7

The validation of nomogram using ROC curves, calibration curve, and DCA curve analysis, respectively. (A) The ROC curves for the prediction of 3-, 6-, 12-month PFS rate of gastrointestinal cancer patients. (B) The calibration curve analysis of the nomogram compared for 3-, 6-, 12-month PFS. (D–F) DCA curve analysis of the nomogram compared for 3-, 6-, 12-month PFS.
Figure 8

(A) Kaplan-Meier curves of PFS in low-risk group, and high-risk group. (B) The AUC values of the nomogram-based risk stratification system for the prediction of 3-, 6-, 12-month PFS rate of gastrointestinal cancer patients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx